

Effects of Two Antioxidants on the Morpho-Biometrical Parameters, Apparent Digestibility and Meat Composition in Rabbits Fed Low and High Fat Diets

¹P.G. Peiretti and ²G. Meineri

¹Institute of Science of Food Production, National Research Council,
Via L. da Vinci, 44-10095, Grugliasco (TO), Italy

²Department of Animal Production, Epidemiology and Ecology,
University of Torino, Via L. da Vinci, 44-10095, Grugliasco (TO), Italy

Abstract: The objective of this study was to determine the effects of the inclusion of Dehydroepiandrosterone (DHEA) and *Spirulina platensis* (SP) in Low Fat (LF) and High Fat (HF) diets on the morpho-biometrical parameters, apparent digestibility and *Longissimus dorsi* (LD) muscle composition of New Zealand White rabbits. The trial was carried out on 24 rabbits that were randomly allocated to six groups of four animals each, kept in individual cages. The three LF diets and the three HF diets (control, with 0.02% DHEA and with 1% SP, respectively) differed on the basis of the level of fat (3 and 13%, respectively). The experimental period lasted 3 months and the feces were collected during the last week. The measured parameters were the initial and final weight, waist circumference and length, digestibility of Dry Matter (DM), Organic Matter (OM), Crude Protein (CP), Ether Extract (EE) and Gross Energy (GE). At the end of the experimental period, the rabbits from each group were slaughtered without fasting and the *Longissimus dorsi* (LD) muscles were collected to determine chemical composition and GE. The results show that the DHEA supplementation in rabbits fed high fat diets decreases EE digestibility, while the SP supplementation increases CP digestibility of low and high fat diets. The DM, OM and GE digestibilities, morpho-biometrical parameters and meat composition were not significantly affected by the type of antioxidant in either the rabbits fed low fat diets or in the rabbits fed high fat diets.

Key words: Rabbit, *Spirulina platensis*, dehydroepiandrosterone, digestibility, acid insoluble ash, meat

INTRODUCTION

There has been an increasing interest in the use of antioxidants in rabbit feed formulas because the dietary manipulation of tissue lipid composition to produce meat with a high PUFA content could decrease meat oxidative stability (Hernandez, 2008).

Antioxidants include endogenously synthesized compounds (Decker *et al.*, 2000) and exogenous substances (Hernandez, 2008). The antioxidant properties of Dehydroepiandrosterone (DHEA), among the endogenous antioxidants (Iwasaki *et al.*, 2004) and some blue-green algae, such as *Spirulina platensis* (SP), among the exogenous antioxidant (Belay, 2002) have recently attracted the attention of researchers in this field due to their potential health benefits.

DHEA is a steroid hormone produced by the adrenal cortex, from the gonads and partly by the brain. In rodents, DHEA has been reported to decrease energy intake and dietary fat as well as body weight and fat content (Abadie *et al.*, 2001; Kajita *et al.*, 2003; Ryu *et al.*, 2003). However, the action mechanisms of DHEA on body

composition are not yet fully understood, although, a role in the utilization or storage of ingested energy (Mohan *et al.*, 1990) and in food intake regulation has been suggested by Pham *et al.* (2000). The anti-obesity and anti-ageing properties of DHEA could be related to a reduction in Crude Protein (CP) digestibility, in the short term and a protective effect on body protein with a selective mass loss from body fat (De Heredia *et al.*, 2007).

SP is used as a therapeutic supplement for the management of various nutritional and metabolic disorders (Henrikson, 1997; Mani *et al.*, 2000) due to its high concentrations of functional nutrients, as it is a rich source of CP, β -carotene, vitamin B₁₂ and essential fatty acids, mainly γ -linoleic acid. SP extracts cause an anti-obesity action (Richmond, 1992), lipid lowering (Iwata *et al.*, 1990), a hypocholesterolemic action (Nakaya *et al.*, 1998) and antioxidant effects (Miranda *et al.*, 1992). In particular, the C-Phycocyanin, pigment of SP has shown antioxidant activity as a scavenger towards free radicals (Lissi *et al.*, 2000; Bhat and Madyastha, 2001).

The addition of fat to rabbit diets improves the energy level of the diet and results in higher digestible energy intake, growth and feed efficiency (Fernandez *et al.*, 1994). If fat addition is low (2.0-6.0%), the digestive use of the entire diet or single nutrient may be improved (Xiccato *et al.*, 1998) and this can improve carcass yield (Beynen *et al.*, 1990). Little research has been done on the effects of high levels of fat in the diets on rabbit digestibility. When fat addition is high (>9%), rabbit carcasses are fatter (Christ *et al.*, 1996), while the water and CP content decreases (Pla and Cervera, 1997).

The main objective of the present study was to examine the effects of DHEA and SP on the digestive efficiency of dietary Dry Matter (DM), Organic Matter (OM), CP, fat and energy in rabbits fed low and high fat diets.

MATERIALS AND METHODS

Animals, diets and housing conditions: The experiment, which lasted 3 months was carried out at the CISRA (Centro Interdipartimentale Servizio Ricovero Animali) experimental rabbitry of the University of Turin. Twenty four adult male New Zealand white rabbits (Harlan, S. Pietro al Natisone, UD, Italy), weighing on average 2807.0±84.0 g, were randomly assigned to 6 groups of four with equal initial weight variability. The rabbits, which were kept in individual cages in an animal room temperature (22±2°C) under a 12 h dark-light cycle, were weighed individually at the beginning and the end of the study. During this period, the rabbits in each group were fed 100 g day⁻¹ of the following diets:

- LF standard rabbit diet
- LF+DHEA standard rabbit diet with dehydroepiandrosterone (0.02%)
- LF+SP standard rabbit diet with *Spirulina platensis* (1%)
- HF high fat diet (standard rabbit diet with 10% added fat)
- HF+DHEA high fat diet with dehydroepiandrosterone (0.02%)
- HF+SP high fat diet with *Spirulina platensis* (1%)

All the experimental diets were prepared by Laboratorio Dottori Piccioni (Gessate, MI, Italy) and formulated to meet all the essential nutrient requirements for rabbits according to the NRC (1977) with the following vitamin and mineral levels recommended for rabbits (per kg diet): vit. A 18000 UI, vit. D₃ 1800 UI, vit. E 40 mg, vit. B₁ 8 mg, vit. B₂ 10 mg, vit. B₆ 8 mg, D-pantothenic acid 10 mg, vit. K 2 mg, niacin 20 mg, vit. B₁₂ 0.2 mg, folic acid 2 mg, choline 1 g, biotin 0.1 mg, DL-methionine 500 mg,

Table 1: Chemical composition (g/100g DM) and gross energy (MJ kg⁻¹ DM) of the Low Fat (LF) control diet and of the Dehydroepiandrosterone (LF+DHEA) and *Spirulina platensis* (LF+SP) supplemented diets

Parameters	LF	LF+DHEA	LF+SP
Dry matter	92.4	92.6	92.6
Organic matter	90.5	90.5	90.6
Crude protein	19.1	17.7	18.4
Crude fibre	14.5	15.3	14.4
Ether extract	3.1	3.1	3.1
Crude ash	9.5	9.5	9.4
Nitrogen free extract	53.8	54.4	54.7
Neutral detergent fibre	34.3	35.4	34.0
Acid detergent fibre	18.5	18.5	18.4
Acid detergent lignin	3.2	3.4	3.1
Gross energy	18.1	17.8	18.5

Table 2: Chemical composition (g/100 g DM) and gross energy (MJ kg⁻¹ DM) of the High Fat control diet (HF) and of the Dehydroepiandrosterone (HF+DHEA) and *Spirulina platensis* (HF+SP) supplemented diets

Parameters	HF	HF+DHEA	HF+SP
Dry matter	94.2	94.1	94.7
Organic matter	91.4	91.5	91.3
Crude protein	16.3	16.3	17.0
Crude fibre	14.9	16.0	15.0
Ether extract	12.5	12.1	13.5
Crude ash	8.6	8.5	8.7
Nitrogen free extract	47.7	47.1	45.8
Neutral detergent fibre	33.0	32.8	32.5
Acid detergent fibre	17.4	17.1	15.9
Acid detergent lignin	3.1	3.1	3.5
Gross energy	19.9	20.6	20.1

J 0.6 mg, Mn 45 mg, Co 0.25 mg, Fe 50 mg, Zn 40 mg and Cu 5 mg. The standard rabbit diet contained the following ingredients: corn, barley, wheat grain, dehydrated alfalfa meal, soybean seed meal, wheat red shorts, carob pulp, torula yeast, dicalcium phosphate, calcium carbonate, salt and magnesium oxide.

These diets were identical in composition, except for the type of antioxidants (DHEA or SP) and for the level of fat (low or high). About 10% of fat, composed of 90% of soybean oil and 10% of lard, was added to the three high fat level diets. All the diets were stored in the dark to avoid auto-oxidation of the lipid sources. During the trials, the 6 diets were sampled monthly to determine the chemical composition and GE (Table 1 and 2).

The rabbits had free access to clean drinking water and throughout the experiments. No obvious health problems were encountered during the experiment and no rabbits died during the feeding trial.

Digestibility assay: The apparent digestibilities of the 6 diets were determined in the last week of the experiment. The feces were collected over a period of 5 days and the collection was performed at approximately 9 h each morning before the next daily ration was provided. The feces were collected using a nylon net placed under each trial cage, to avoid urine contamination. Each pooled fecal

sample was taken and placed in a two-layer plastic bag to prevent the loss of moisture. The frozen samples were individually mixed thoroughly and pooled, ground in a homogenizer (Tecator, Herndon, VA, USA) and the representative samples were then weighed on an aluminum foil pan, dried in a draft oven at 80°C to constant weight and stored for chemical analysis.

The digestibility coefficients were calculated through standard procedures, following the indirect digestibility method (Furuichi and Takahashi, 1981), using Acid Insoluble Ash (AIA) as an inert marker.

Morpho-biometrical parameters: At the end of the experimental period, the rabbits from each group were weighed and measured for waist circumference and length and then slaughtered without fasting. A sample of *Longissimus dorsi* (LD) muscle was excised 24 h post mortem from the carcass and immediately frozen at -20°C until the analysis.

Analytical determinations: All the analysis were carried out on triplicate samples. The proximate composition of the LD muscles, diets and feces was determined according to the AOAC (1990) method. The samples were analyzed to determine: DM, total N content, ash by ignition to 550°C and Ether Extract (EE) using the Soxhlet method, while the Gross Energy (GE) was determined using an adiabatic calorimeter bomb (IKA C7000, Staufen, Germany). Neutral Detergent Fibre (NDF) and Acid Detergent Fibre (ADF) were determined on the diets and feces, as described by Van Soest *et al.* (1991). The acid insoluble ash content of the diets and faeces was determined according to Van Keulen and Young (1977).

Statistical analysis: Statistical analysis were performed using the SPSS software package (version 11.5.1 for Windows, SPSS Inc., USA). Analysis of variance was used to evaluate the effects of DHEA or SP supplementation on some morpho-biometrical parameters, apparent digestibility coefficients, chemical composition and GE of the LD muscle, respectively. The differences were tested separately for the Low Fat (LF) groups and High Fat (HF) groups of the animals and the means were compared with a Duncan test at a significance level of 5%.

RESULTS AND DISCUSSION

Morpho-biometrical parameters: The final body weight, waist circumference and length of the rabbits fed the low and high fat diets were not significantly affected by the type of antioxidant (Table 3 and 4, respectively). These

Table 3: Initial and final weight (g), waist circumference and length (cm) of rabbits fed Low Fat control diet (LF) and of the Dehydroepiandrosterone (LF+DHEA) and *Spirulina platensis* (LF+SP) supplemented diets¹

Parameters	LF	LF+DHEA	LF+SP
Initial weight	2791±22	2772±63	2794±53
Final weight	3155±58	3024±104	3134±55
Waist circumference	33.0±0.4	31.3±0.9	33.3±0.7
Length	52.8±0.5	52.2±0.7	52.6±0.4

Table 4: Initial and final weight (g), waist circumference and length (cm) of rabbits fed High Fat (HF) control diet and of the Dehydroepiandrosterone (HF+DHEA) and *Spirulina platensis* (HF+SP) supplemented diets¹

Parameters	HF	HF+DHEA	HF+SP
Initial weight	2836±14	2837±71	2799±36
Final weight	3535±75	3521±105	3374±75
Waist circumference	37.5±0.6	35.8±0.2	36.8±0.9
Length	54.0±0.8	54.0±0.7	51.8±0.2

¹The data (n = 4 for each group) are the means±SE

results are in agreement with other studies that found no effect of DHEA on body weight (Lea-Currie *et al.*, 1997a, b; Aragno *et al.*, 2004). However, de Heredia *et al.* (2007) found an immediate slimming effect of a DHEA treatment in high fat fed rats, in agreement with other studies, which show the anti-obesity properties of this hormone (Tagliaferro *et al.*, 1995). These differences could be a consequence of the length of the treatment or of the different amounts of DHEA supplemented in the diets. The DHEA dosage used in our study was lower than that used by de Heredia *et al.* (2007).

As far as the SP supplementation is concerned, previous studies evaluated higher inclusions of this micro algae as a protein replacement for soybean meal in rabbits, chickens and pigs. Peiretti and Meineri (2008) shown that the final weight, weight gain and feed efficiency did not differ significantly in growing rabbits fed diets with increasing levels of SP (5, 10 and 15%), but an SP inclusion level of 10% gave the highest feed intake. Yoshida and Hoshi (1980) fed varying levels of spirulina to growing chickens with satisfactory results at the lower levels of 5.0-10.0%; growth was depressed at levels above 20.0%. Grinstead *et al.* (2000) reported inconsistent and minimal improvement in growth performance when pigs were fed diets containing 0.2, 0.5 and 2.0% SP.

Digestibility assay: The apparent digestibilities of DM, OM and GE were not affected by the type of antioxidant, in either the rabbits fed low fat diets or in the rabbits fed high fat diets. The data shows that CP digestibility was higher in the SP supplemented group than in the other two groups in both the rabbits fed low fat diets and in the rabbits fed high fat diets (Table 5 and 6, respectively).

As far as EE digestibility is concerned, no significant differences were found in rabbits fed low fat diets between the two antioxidant groups and the control group

Table 5: Percent of the apparent digestibility coefficients (means±SE) of the Low Fat diets (LF) control diet and of the Dehydroepiandrosterone (LF+DHEA) and *Spirulina platensis* (LF+SP) supplemented diets¹

Parameters	LF	LF+DHEA	LF+SP
Dy matter	62.9±0.6	62.1±0.8	64.0±0.6
Organic matter	64.7±0.6	63.7±0.8	65.9±0.7
Crude protein	69.8±1.0 ^a	70.9±0.9 ^{ab}	73.2±0.4 ^b
Ether extract	86.9±0.9	88.1±1.0	88.4±1.2
Gross energy	63.8±0.9	62.2±0.9	65.2±0.8

Table 6: Percent of the apparent digestibility coefficients (means±SE) of the High Fat control diet (HF) and of the Dehydroepiandrosterone (HF+DHEA) and *Spirulina platensis* (HF+SP) supplemented diets¹

Parameters	HF	HF+DHEA	HF+SP
Dy matter	65.5±0.4	63.0±1.0	65.3±0.4
Organic matter	67.2±0.4	65.3±0.8	67.0±0.4
Crude protein	70.8±0.2 ^a	70.7±0.6 ^a	72.5±0.3 ^b
Ether extract	94.2±0.2 ^a	93.1±0.2 ^b	93.7±0.1 ^{ab}
Gross energy	68.4±0.6	67.9±0.7	68.7±0.1

¹Values (n = 4 for each group) in the same row with different superscripts differ (p<0.05) and were obtained using acid insoluble ash as the internal marker

while in rabbits fed high fat diets, the EE digestibility was significantly lower in the DHEA supplemented group than in the other two groups.

The EE digestibilities of all the high fat diets were higher than those of the low fat diets with increments that ranged from 5.0% in the HF+DHEA diet to 7.3% in the HF control diet, while the CP digestibilities of the high fat diets were always similar to those of the low fat diets.

In aged rats fed a high fat diet, De Heredia *et al.* (2007) demonstrated that DHEA exerts a specific action at a digestive level. These researchers suggest that the anti-obesity and anti-ageing properties of DHEA could be related to a reduction in CP digestibility and a protective effect on body protein, with a selective mass loss from body fat and the DHEA properties varying according to the length of treatment.

Peiretti and Meineri (2008) shown that the DM, OM, CP and GE digestibilities of SP diets were lower than those of the control diet. A 15% inclusion of SP in the diet resulted in very low digestibilities compared to the control and compared to diets containing 5 and 10% of SP and the differences were significant in each case. The only exception was the CP digestibility, which only differed from that of the control diet. The EE digestibility of the group fed the 10% SP diet was similar to that of the control group and was higher than the other two SP groups.

Muscular composition: The different types of antioxidant did not influence the chemical composition or GE of the LD muscle of rabbits fed low or high fat diets (Table 7 and 8, respectively). Peiretti and Meineri (2009) reported that

Table 7: Chemical composition (g/100 g DM) and gross energy (MJ kg⁻¹ DM) of the *longissimus dorsi* muscle of rabbits fed Low Fat (LF) control diet and of the Dehydroepiandrosterone (LF+DHEA) and *Spirulina platensis* (LF+SP) supplemented diets after 1 day of storage at 4°C¹

Parameters	LF	LF+DHEA	LF+SP
Dry matter	26.0±0.4	25.6±0.3	25.5±0.60
Crude protein	91.7±0.9	91.9±1.6	91.2±1.20
Ether extract	2.8±0.6	2.0±0.5	2.7±0.90
Ash	4.8±0.1	4.7±0.1	4.8±0.10
Gross energy	22.0±0.2	21.5±0.4	22.1±0.20

Table 8: Chemical composition (g/100 g DM) and gross energy (MJ kg⁻¹ DM) of the *longissimus dorsi* muscle of rabbits fed High Fat (HF) control diet and of the Dehydroepiandrosterone (HF+DHEA) and *Spirulina platensis* (HF+SP) supplemented diets after 1 day of storage at 4°C¹

Parameters	HF	HF + DHEA	HF + SP
Dry matter	27.6±0.4	26.9±0.3	26.9±0.1
Crude protein	89.5±0.6	89.5±0.8	89.9±0.5
Ether extract	3.9±0.8	4.1±0.6	4.2±0.1
Ash	4.3±0.1	4.6±0.2	4.5±0.1
Gross energy	22.4±0.2	22.6±0.3	22.6±0.1

¹The data (n = 4 for each group) are the means±SE

DHEA does not influence the FA profile of perirenal fat or the LD muscle of rabbits fed diets with different fat contents, but its action is more aimed at a quantitative reduction of the fat deposition.

CONCLUSION

The present results show that a 0.02% DHEA supplementation in rabbits fed high fat diets decrease EE digestibility. It can therefore be suggested that the anti-obesity property of DHEA could also be related to a reduction in EE digestibility. SP supplementation at a 1% level only increased the CP digestibility of low and high fat diets. The DM, OM and GE digestibilities, morpho-biometrical parameters and meat composition were not significantly affected by the type of antioxidant.

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