

## Fat and Meat Fatty Acid Profile of Rabbits Fed Different Fat Content and Dehydroepiandrosterone Supplementation Diets

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**Abstract:** The aim of the present study was to examine the effects of Dehydroepiandrosterone (DHEA) on the perirenal fat and Longissimus Dorsi (LD) muscle Fatty Acid (FA) profile of rabbits fed diets with low (3%) and high fat (13%) contents. DHEA was given in the diet (0-0.02%) to 15-week-old male New Zealand White rabbits for 3 months. At the end of the experimental period, the rabbits from each group were slaughtered without fasting and the perirenal fat and LD muscle were collected to determine the FA profile. The results show that DHEA, at the experimental dosage, does not influence the FA profile of perirenal fat or LD muscle of rabbits fed different fat content diets.

**Key words:** Rabbit, fat, muscle, Dehydroepiandrosterone, fatty acid

### INTRODUCTION

The composition of the dietary fat, as a result of the use of various fat sources, can considerably modify the Fatty Acid (FA) composition of the fatty tissues of rabbit (Dalle, 2002). Cobos *et al.* (1993) noted that enriching the diet of rabbits with soya, sunflower oils or soya bean oil increased the proportion of unsaturated FA compared to those obtained using conventional diets and this was considered an important nutritional benefit for the consumer. The inclusion of a dietary fat content in moderate concentrations (3-6%) can improve carcass yield (Dalle, 2002). If the dietary fat supplement is increased beyond these values, the fat deposition also increases (Oliver *et al.*, 1997). However, when higher amounts of fat are included, the fat content of the meat increases (Dalle, 2002).

Dehydroepiandrosterone (DHEA) and its sulfate are the most abundant circulating steroids in man and they are known as “the hormones of youth” because their serum concentration decreases gradually with ageing and coincides with the incipient loss of physical performance (Nawata *et al.*, 2002). The decline in DHEA concentration in humans during ageing is usually associated with the development of obesity (Yamaguchi *et al.*, 1998).

The results of a study by Hansen *et al.* (1997) in rats show that the administration of DHEA to a large extent protects against the development of visceral obesity which is associated with high fat feeding. In rodents,

DHEA has been reported to decrease dietary fat and energy intakes as well as body weight and fat content (Pham *et al.*, 2000; Abadie *et al.*, 2001; Kajita *et al.*, 2003; Ryu *et al.*, 2003).

Studies exist on the direct effects of DHEA on muscle, which suggest another mechanism for the hormone action on body composition (Campbell *et al.*, 2004). Kochan and Karbowska (2004) hypothesised that the fat reducing effect of DHEA on adipose tissue might be exerted due to the influence of this hormone on Peroxisome Proliferator Activated Receptor (PPAR $\alpha$ ) gene expression. PPAR $\alpha$ , a nuclear hormone receptor, has been found to regulate the expression of peroxisomal, mitochondrial and microsomal enzymes involved in the  $\beta$ -oxidation of FAs (Brandt *et al.*, 1998; Muoio *et al.*, 2002). Since, the FA oxidation pathway in rodents is under PPAR $\alpha$  control, which regulates the transcription of the gene encoding enzyme involved in FA catabolism, an increase in PPAR $\alpha$  concentration in adipose tissue may result in an increase in the rate of lipolysis and FA oxidation in adipocytes and subsequently, a decrease in fat mass after DHEA treatment. An increase in FA utilisation by adipose tissue should result in a reduction in adipose tissue mass. This reduction in adipose tissue mass occurred although there was no difference in food intake, a result which is consistent with other studies (Cleary *et al.*, 1985). FAs are metabolized in the liver by  $\beta$ -oxidation in mitochondria and peroxisomes and by  $\omega$ -oxidation in microsomes. Mitochondrial  $\beta$ -oxidation is

responsible for the oxidation of short, medium and long chain FAs and peroxisomal  $\beta$ -oxidation is responsible for the metabolism of very long chain FAs. Very long chain FAs are also metabolized by the cytochrome P450 CYP4A  $\omega$ -oxidation system to dicarboxylic acids (Yu *et al.*, 2003).

However, the mechanisms of action of this hormone on body composition are not yet fully understood and no specific studies have been found in literature that have focused on the influence of DHEA on the FA profile of rabbit tissues.

The objective of this study was to determine the effects of DHEA on the fat and meat FA profile of rabbits fed diets with different fat contents.

## MATERIALS AND METHODS

The study was carried out at the CISRA (Centro Interdipartimentale Servizio Ricovero Animali) experimental rabbitry of the University of Turin. Male New Zealand White rabbits (Harlan-Italy, Udine, Italy), weighing on average  $2812 \pm 86$  g were cared with the Principles of Laboratory Animal Care (NIH no. 85-23, revised 1985) and randomly assigned to 4 groups with equal initial weight variability. The animals were kept in individual cages in an animal room (temperature  $22 \pm 2^\circ\text{C}$ ) under a 12 h dark-light cycle with free access to clean drinking water and were allowed an adaptation period of 2 weeks.

The rabbits of each group were fed 100 g of the following diets/day:

- Low Fat Control (LF) group (n = 4): received a standard rabbit diet
- Low Fat DHEA (LF + DHEA) group (n = 4): received a standard rabbit diet supplemented with DHEA (0.02%)
- High Fat (HF) group (n = 4): received a high fat diet consisting of a standard rabbit diet with 10% added fat (6.7% corn oil and 3.3% lard)
- High Fat DHEA (HF + DHEA) group (n = 4): received a high fat diet supplemented with DHEA (0.02%)

All the experimental diets were prepared by Laboratorio Dottori Piccioni (Gessate, MI, Italy), were formulated to meet all the essential nutrient requirements for rabbits according to the National Research Council (1977) and contained the recommended levels of vitamins and minerals for rabbits. 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 DHEA

supplementation (0 and 0.02%) and level of fat (3 and 13%). During the trials, the 4 diets were sampled to determine the chemical composition, Gross Energy (GE) and FA profile.

At the end of the experimental period, which lasted 3 months, the rabbits from each group were slaughtered without fasting. The Longissimus Dorsi (LD) muscle and perirenal fat samples were collected 24 h post mortem from the carcass and immediately frozen at  $-20^\circ\text{C}$  until the analyses.

All the analyses were carried out on duplicate samples. The proximate composition of the diets were determined according to the AOAC (1990). The diet samples were analysed to determine: dry matter, total N content, ash by ignition to  $550^\circ\text{C}$  and ether extract using the Soxhlet method, crude fibre according to the Weende method, neutral detergent fibre without sodium sulfite and  $\alpha$ -amylase and acid detergent fibre, as described by Van Soest *et al.* (1991) expressed exclusive of residual ash. Acid detergent lignin was determined by solubilization of cellulose with sulphuric acid, as described by Robertson and Van Soest (1981) and GE using an adiabatic calorimeter bomb (IKA C7000, Staufen, Germany).

Lipid extraction was performed on the diets and the meat and fat samples according to Hara and Radin (1978), while the transesterification of the FAs was carried out according to Christie (1982), with the modifications described by Chouinard *et al.* (1999).

The FAs were analysed as their methyl esters. The analysis was carried out by gas chromatography, using a Dani GC 1000 DPC (Dani Instruments S.P.A., Cologno Monzese, Italy), equipped with a fused silica capillary column-Supelcowax-10 ( $60 \text{ m} \times 0.32 \text{ mm}$  (i.d.),  $0.25 \mu\text{m}$ ). The PTV injection and Flame Ionization Detector (FID) ports were set at  $245$  and  $270^\circ\text{C}$ , respectively. The oven temperature program was initially set at  $50^\circ\text{C}$  for the first min and then increased at a rate of  $15^\circ\text{C min}^{-1}$  to  $200^\circ\text{C}$ , where it remained for 20 min and then increased at a rate of  $5^\circ\text{C min}^{-1}$  to  $230^\circ\text{C}$ , where it remained for the last 3 min. The carrier gas was hydrogen. One microlitre was injected using a Dani ALS 1000 auto sampler with a split ratio 1:50. The peak area was measured using a Dani Data Station DDS 1000 and each peak was identified and quantified by pure methyl ester standards (Restek Corporation, Bellefonte, PA, USA).

Statistical analyses were performed using the SPSS software package (version 11.5.1 for Windows, SPSS Inc., USA). The analysis of variance was used to evaluate the effects of DHEA on the FA profiles of the meat and perirenal fat of the rabbits, respectively. The differences among the FA profiles of the meat and perirenal fat of the rabbits were tested separately for Low Fat (LF) groups and High Fat (HF) groups of the animals using the t-test.

**RESULTS AND DISCUSSION**

The chemical composition and GE of the four diets are reported in Table 1. The two LF diets differed from the two HF diets in ether extract, nitrogen free extract and GE content, due to the addition of 10% of lipids (6.7% corn oil and 3.3% lard) to the two HF diets.

The FA pattern of the diets (Table 2) was characterized by higher percentages of linoleic acid (LA, C18:2n-6) and  $\alpha$ -linolenic acid (LNA, C18:3n-3) and lower percentages of stearic acid (SA, C18:0) and oleic acid (OA, C18:1) in the LF diets than in the HF diets. Consequently, the LF diets presented a lower MUFA and SFA percentages and a higher PUFA percentage than the HF diets. Moreover, the n-6/n-3 ratio in the two HF diets was higher than that of two LF diets, due to their low content of LNA.

The FA composition in the LD muscle of the rabbits is reported in Table 3. The FA profile of the muscle of the rabbits fed the LF diets was characterized by a lower percentages of OA and LA and a higher percentages of palmitic acid (PA, C16:0) and SA than the muscle of the rabbits fed the HF diets. Consequently, the muscle of the rabbits fed the LF diets presented a lower PUFA percentage and higher SFA percentage than the muscle of the rabbits fed the HF diets. As far as the effect of DHEA is concerned, there were no significant differences in the muscle FAs of the rabbits fed diets with or without DHEA.

The FA profile of the perirenal fat of the rabbits, reported in Table 4, was similar to that of the muscle with low percentages of OA and LA in all the rabbit groups. Consequently, the perirenal fat of the rabbits presented a higher PUFA percentage and lower MUFA and SFA percentages than the muscle of the corresponding rabbits. As far as the effect of DHEA is concerned, there were no significant differences in the perirenal fat FAs of the rabbits fed diets with or without DHEA.

The DHEA dosage (0.02%) used in our study was lower than that used by Abadie *et al.* (2001). These authors hypothesised that DHEA may alter long-chain FA profiles in muscle tissue of obese rats and they therefore more closely resemble that of lean rats. The DHEA functions are related to their effects on the FA profile. It has been demonstrated that a DHEA treatment in obese rats significantly increases the hepatic tissue proportion of PUFA (Abadie *et al.*, 2000).

The mechanisms by which DHEA acts on body composition still remains to be clarified. Various authors have suggested different targets for the anti-obesity properties of DHEA, such as changes in the central regulation of food intake (Tagliaferro *et al.*, 1986; Svec and Porter, 1997; Gillen *et al.*, 1999), increases in thermogenesis in white and brown adipose tissues

Table 1: Chemical composition (g/100 g DM) and gross energy (MJ kg<sup>-1</sup> DM) of the Low Fat (LF) and High Fat (HF) diets with or without dehydroepiandrosterone (DHEA) supplementation

	LF	LF + DHEA	HF	HF + DHEA
Dry matter	92.4	92.6	94.2	94.1
Organic matter	90.5	90.5	91.4	91.5
Crude protein	19.1	17.7	16.3	16.3
Crude fibre	14.5	15.3	14.9	16.0
Ether extract	3.1	3.1	12.5	12.1
Crude ash	9.5	9.5	8.6	8.5
Nitrogen free extract	53.8	54.4	47.7	47.1
Neutral detergent fibre	34.3	35.4	33.0	32.8
Acid detergent fibre	18.5	18.5	17.4	17.1
Acid detergent lignin	3.2	3.4	3.1	3.1
Gross energy	18.1	17.8	19.9	20.6

Vitamins and minerals per kg diet: Vit. A 18000UI; Vit. D<sub>3</sub> 1800UI; Vit. E 40 mg; Vit. B<sub>1</sub> 8 mg; Vit. B<sub>2</sub> 10 mg; Vit. B<sub>6</sub> 8 mg; D-pantothenic Acid 10 mg; Vit. K 2 mg; Niacin 20 mg; Vit. B<sub>12</sub> 0.2 mg; Folic Acid 2 mg; Choline 1 g; Biotin 0.1 mg; DL-methionine 500 mg; J 0.6 mg; Mn 45 mg; Co 0.25 mg; Fe 50 mg; Zn 40 mg; Cu 5 mg

Table 2: Fatty acid (FA) pattern of the Low Fat (LF) and High Fat (HF) diets (g/100 g of total FA) with or without dehydroepiandrosterone (DHEA) supplementation

	LF	LF + DHEA	HF	HF + DHEA
C14:0	0.3	0.2	0.5	0.6
C16:0	15.7	14.9	15.7	16.2
C16:1	0.3	0.3	0.8	0.8
C17:0	0.7	0.4	0.2	0.2
C17:1	0.0	0.0	0.1	0.0
C18:0	2.4	2.1	5.8	6.4
C18:1	21.3	19.7	31.7	31.7
C18:2n-6	50.7	51.5	41.2	40.1
C18:3n-3	7.3	9.5	2.3	2.3
C20:0	0.0	0.3	0.3	0.3
C20:1n-9	0.6	0.6	0.5	0.5
C20:2n-9	0.0	0.0	0.3	0.2
Other	0.7	0.6	0.7	0.7
SFA <sup>1</sup>	19.1	17.8	22.5	23.7
MUFA <sup>2</sup>	22.1	20.6	33.0	33.0
PUFA <sup>3</sup>	58.0	61.0	43.7	42.6
n-6/n-3 <sup>4</sup>	6.9	5.4	17.9	17.4

SFA<sup>1</sup>: Saturated Fatty Acid, MUFA<sup>2</sup>: Monounsaturated Fatty Acid, PUFA<sup>3</sup>: Polyunsaturated Fatty Acid, n-6/n-3<sup>4</sup>: PUFA n-6/PUFA n-3 ratio

Table 3: Effects of diet on Fatty Acid (FA) composition (g/100 g of total FA) in the *longissimus dorsi* muscle of rabbits fed Low Fat (LF) and High Fat (HF) diets with or without Dehydroepiandrosterone (DHEA) supplementation<sup>1</sup>

	LF	LF + DHEA	HF	HF + DHEA
C14:0	2.30±0.30	2.18±0.04	1.62±0.09	1.58±0.09
C14:1	0.33±0.10	0.30±0.02	0.26±0.07	0.27±0.07
C15:0	0.45±0.03	0.42±0.01	0.31±0.02	0.28±0.01
C16:0	26.13±0.82	26.27±0.18	21.26±0.46	21.44±1.61
C16:1	5.78±1.02	5.51±0.27	4.34±0.54	4.59±0.32
C17:0	0.56±0.02	0.53±0.02	0.37±0.03	0.38±0.01
C17:1	0.41±0.04	0.40±0.01	0.27±0.01	0.29±0.03
C18:0	6.10±0.23	6.19±0.24	5.43±0.06	5.76±0.31
C18:1	28.68±0.69	28.65±0.46	31.81±0.38	31.56±0.98
C18:2n-6	20.56±1.24	19.52±1.08	28.67±0.69	27.47±1.77
C18:3n-3	2.43±0.34	2.51±0.67	1.88±0.06	1.87±0.21
C21:0	4.52±1.57	4.92±0.92	2.38±0.25	3.33±1.39
Other	1.75±0.64	2.61±1.08	1.20±0.41	1.18±0.21
SFA <sup>2</sup>	40.07±0.99	40.51±0.46	31.39±0.48	32.78±2.76
MUFA <sup>3</sup>	35.20±1.64	34.86±0.21	36.67±0.68	36.71±0.93
PUFA <sup>4</sup>	22.98±1.11	22.03±1.75	30.75±0.74	29.34±1.97
n-6/n-3 <sup>5</sup>	8.61±1.39	8.01±1.70	15.35±0.16	14.74±0.86
S/P <sup>6</sup>	0.59±0.02	0.61±0.01	0.42±0.01	0.44±0.04

<sup>1</sup>The data are means (n = 4 for each group) ±SD, SFA<sup>2</sup>: Saturated Fatty Acid, MUFA<sup>3</sup>: Monounsaturated Fatty Acid, PUFA<sup>4</sup>: Polyunsaturated Fatty Acid, n-6/n-3<sup>5</sup>: PUFA n-6/PUFA n-3 ratio, S/P<sup>6</sup>: Saturated Fatty Acid/Unsaturated Fatty Acid

Table 4: Effects of diet on Fatty Acid (FA) pattern (g/100 g of total FA) of the perirenal fat of rabbits fed Low Fat (LF) and High Fat (HF) diets with or without Dehydroepiandrosterone (DHEA) supplementation<sup>1</sup>

	LF	LF + DHEA	HF	HF + DHEA
C10:0	0.03±0.01	0.02±0.01	0.02±0.01	0.01±0.01
C14:0	2.03±0.12	2.07±0.11	1.33±0.23	1.13±0.06
C14:1	0.16±0.04	0.15±0.04	0.08±0.03	0.07±0.01
C15:0	0.59±0.04	0.56±0.03	0.33±0.03	0.28±0.01
C16:0	25.64±0.77	25.95±1.15	20.17±1.54	18.70±0.36
C16:1	3.08±0.47	2.98±0.48	2.01±0.46	1.70±0.17
C17:0	0.73±0.06	0.71±0.02	0.43±0.03	0.41±0.01
C17:1	0.34±0.03	0.32±0.02	0.24±0.02	0.20±0.02
C18:0	6.90±0.51	6.85±0.30	6.37±0.62	6.90±0.26
C18:1	24.83±0.46	25.49±1.33	30.74±0.83	30.74±0.19
C18:2n-6	28.76±1.83	27.59±1.81	32.9±1.42	34.67±0.69
C18:3n-3	3.37±0.23	3.89±0.33	2.43±0.20	2.24±0.20
C18:3n-6	0.22±0.04	0.21±0.04	0.12±0.01	0.12±0.02
C20:0	0.19±0.05	0.16±0.05	0.15±0.02	0.15±0.02
C20:1n-9	0.45±0.08	0.44±0.15	0.44±0.06	0.48±0.17
C20:2n-6	0.25±0.03	0.25±0.02	0.29±0.04	0.28±0.04
C20:4n-6	0.06±0.02	0.07±0.01	0.05±0.00	0.04±0.03
C21:0	0.13±0.02	0.11±0.02	0.11±0.00	0.09±0.03
Other	2.25±0.18	2.18±0.05	1.77±0.07	1.79±0.38
SFA <sup>2</sup>	36.22±0.91	36.42±0.95	28.92±1.92	27.67±0.55
MUFA <sup>3</sup>	31.95±1.33	32.36±2.00	35.52±1.30	34.88±1.19
PUFA <sup>4</sup>	32.66±1.73	32.01±1.89	35.8±1.23	37.35±1.16
n-6/n-3 <sup>5</sup>	8.75±1.08	7.26±0.82	13.81±1.64	15.79±2.00
S/P <sup>6</sup>	0.53±0.02	0.54±0.02	0.39±0.04	0.37±0.01

<sup>1</sup>The data are means (n = 4 for each group) ±SD, SFA : Saturated Fatty Acid, MUFA<sup>3</sup>: Monounsaturated Fatty Acid, PUFA<sup>4</sup>: Polyunsaturated Fatty Acid, n-6/n-3<sup>5</sup>: PUFA n-6/PUFA n-3 ratio, S/P<sup>6</sup>: Saturated Fatty Acid/Unsaturated Fatty Acid

(Ryu *et al.*, 2003) and alteration of pre-adipocyte proliferation and differentiation (Lea-Currie *et al.*, 1998). The anti-obesity and antiageing properties of DHEA could be related to a reduction in protein digestibility and a protective effect on body protein, with a selective mass loss from body fat and that the properties of DHEA vary according to the treatment length. De Heredia *et al.* (2007) showed that DHEA decreased energy intake, body weight, body fat and adipocyte size and number. The feed efficiency ratio indicates that DHEA-treated rats were less efficient in transforming nutrients fed into their own biomass. A short-term reduction in protein digestibility and in body-protein degradation was also found for the DHEA-treated rats, resulting in an increased content of body protein.

### CONCLUSION

This research has shown that DHEA, at the experimental dosage, 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. Other metabolic mechanisms regarding the action of the absorption of the lipidic fraction should be checked, particularly PPARS activation and the reduction of Reactive Oxygen Species (ROS), which, based on recent studies, influence the deposition of fat in the adipose tissues.

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