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DIGESTIBILITY OF NUTRIENTS WITH THE INCLUSION OF CONJUGATED LINOLEIC ACID IN THE DIET OF DOGS

(Digestibilidade dos nutrientes com a inclusão de ácido linoléico conjugado na dieta de cães)

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ABSTRACT - The inclusion of the conjugated linoleic acid (CLA) in food for dogs has been displaying great potential, taking into account several benefits in regard to the use of this product. To determine the effect of CLA on the digestibility of the dry matter, nutrients and energy in dogs, two experiments were performed using dogs of Beagle type, in the age of seven and 15 months, respectively. The experimental diets were isonutritive, just differing by the inclusion of CLA, watching for the addition to the control-diet, of an amount of lipids in the form of chicken fat equivalent to the amount of CLA added (0.3%), so that the diets were isoenergetic. The coefficients of apparent digestibility of the dry matter, crude protein, ethereal extract and nitrogen-free extract were not affected by the inclusion of conjugated linoleic acid, showing that the addition of the product did not interfere with the process of digestion of nutrients. In both digestibility experiments, higher values of metabolizable energy were observed for the diets with inclusion of CLA ($P < 0.01$) when compared with the control-diet.

Key-words: beagle; CLA; metabolizable energy.

RESUMO - A inclusão do ácido linoléico conjugado (CLA) em rações para cães tem apresentado grande potencial, tendo em vista os vários benefícios relacionados à utilização deste produto. Para determinar o efeito do CLA sobre a digestibilidade da matéria seca, nutrientes e energia em cães foram realizados dois ensaios utilizando cães da raça Beagle, com sete e 15 meses de idade. As dietas experimentais eram isonutritivas, diferindo apenas quanto à inclusão de CLA, tomando-se o cuidado de acrescentar à dieta controle uma quantidade de lipídeos na forma de gordura de frango equivalente à quantidade de CLA adicionada (0,3%), para que as dietas fossem isoenergéticas. Os coeficientes de digestibilidade aparente da matéria seca, proteína bruta, extrato etéreo e extrativos não nitrogenados não foram afetados pela inclusão de ácido linoléico conjugado, mostrando que a adição do produto não interferiu no processo de digestão dos nutrientes. Em ambos os ensaios de digestibilidade, observaram-se valores de energia metabolizável superiores para as dietas com inclusão de CLA ($P<0,01$) quando comparada com a dieta controle.

Palavras-chave: beagle; CLA; energia metabolizável.

INTRODUCTION

Usually, the labels of dogs' food bring values of the minimum nutritional composition (crude protein, ethereal extract and phosphorus) and maximum nutritional composition (humidity, crude fiber, ashes or mineral matter and calcium). However, they do not have data regarding the digestibility of nutrients and energy, which are supplementary results to the chemical composition and of fundamental importance to the nutritional evaluation of the food (LÔBO JR. *et al.,* 2001).

The conjugated linoleic acid (CLA) is a term used to designate a mixture of geometric and positional isomers of the linoleic acid ($C_{18:2}$), which contains two double conjugated bonds (DONOVAN et al., 2000). Researches about the functions and benefits of CLA started about 1980 with the observation that the isolated CLA of beef, or the catalyzed isomerization of the linoleic acid, chemically inhibited the induction of skin neoplasia in mice. Such discovery stimulated other studies in

order to evaluate the beneficial effects of CLA on cancer, immune function, arteriosclerosis, weight gain, energy and food intake, as well as, body composition (WANG and JONES, 2004).

Some works have been showing smaller deposition of body fat, in animal and human models receiving diets supplemented with CLA. The increase of the energy metabolism induced by CLA is one of the several mechanisms suggested to explain the reduction of the body fat (CHOI *et al*., 2004).

WEST *et al.* (2000) using mice with strong susceptibility to obesity, fed with a high-lipid diet with or without CLA at 1% of the dry matter of the diet (with predominance of the *cis*-9, *trans*-11 and *trans*-10, *cis*-12 isomers) for five weeks, have verified reduction of 50% on the weight of the fatty tissue of the animals fed with CLA, when compared to the control group. The final body weight, however, was similar, suggesting the increase of the lean mass and the reduction of the fatty tissue in the animals supplemented with CLA.

The objective of this work is to evaluate the effect of the supply of conjugated linoleic acid on the digestibility of dry matter, nutrients and energy in dogs of Beagle type at the age between seven and 15 months.

MATERIAL AND METHODS

The experiment was developed at the Laboratory of Studies of Canine Nutrition, of the Departamento de Zootecnia da Universidade Federal do Paraná. The experiment was approved by the Committee of Ethics in Research with Animals of PUCPR, under registration at CEPA/PUCPR No. 23. Diets with and without addition of CLA were appraised, and Beagle dogs originated from three broods with approximate dates of birth were used.

The ingredients used in the formulation of the diets used in the two experiments were the same ones, except for the CLA that was added only to the CLA diet (TABLE 1). The chemical composition of the experimental diets is shown on Table 2. The product LUTA-CLA 60 was used as CLA source, which is constituted by colorless liquid oil of vegetable origin (sunflower oil), composed of 60% of conjugated linoleic acid methyl ester, at the proportion of 1:1 of the *cis*-9, *trans*-11: *trans*-10, *cis*-12 isomers.

The inclusion of chicken oil to the control food and CLA to the test diet was accomplished by double cone type mixer (35 rpm) during 15 minutes to mix 75 kg of food. The control food was mixed before the CLA food.

TABLE 1 - INGREDIENTS USED IN THE FORMULATION OF THE EXPERIMENTAL DIETS.

Ingredient	Control	CLA
	Kg/t	
Corn	150	150
Soybean meal	70	70
Rice	320	320
Brewer's Yeast	10	10
Fish Meal	45	45
Chicken Meal	320	320
Corn Oil	10	10
Whole Powdered Milk	9	9
Salt	4.56	4..56
Garlic Flavor	0.04	0.04
Vitamin C	0.15	0.15
Powdered Milk Flavor	5.25	5.25
Antioxidant	0.20	0.20
Yucca	0.25	0.25
Dried Beet Pulp	3	3
Micotoxins Adsorbent	0.50	0.50
Vitamin / Mineral Premix	5	5
Powdered Egg	8	8
Plasma	8	8
Complexed Minerals	0.5	0.5
Mannanoligosaccharides	1.0	1.0
Poultry Fat	30	25
LUTA-CLA 60	0	5

Control = Diet with no inclusion of CLA; CLA = Inclusion of 1% of CLA to the diet (% MS).

TABLE 2 - CHEMICAL COMPOSITION OF THE EXPERIMENTAL DIETS.

Chemical composition (%)	Diet	
	Control	CLA
DM	91.58	91.28
	% MS	
CP	26.77	26.32
EE	9.52	9.58
CF	1.09	1.23
MM	9.97	9.84
Ca	2.70	2.60
P	1.32	1.32
NFE	44.23	44.31

Control = Diet with no inclusion of CLA; CLA = Inclusion of 1% of CLA to the diet (% MS).
DM = Dry matter; CP = Crude protein; EE = Ether extract; CF = Crude fiber; MM = Mineral matter; Ca = Calcium, P = Phosphorus, NFE = nitrogen-free extract.

TABLE 3 - ENERGY NEEDS FOR DOGS IN DIFFERENT STAGES OF LIFE.

Stage	Energy needs (EN)
40% of adult weight	1.6 x adult EN
80% of adult weight	1.2 x adult EN
Low room temperature	1.2 to 1.8 x adult EN

Adapted from FRAGA (2001).

The water was supplied *ad libitum*, while the feeding during the whole experimental period was restricted, in accordance with the body weight of each animal, supplied twice a day, at 10 a.m. and 6 p.m., with no occurrence of orts. The amount supplied per animal was calculated with base on the daily energy need for maintenance, calculated using the equation 132 x body weight^{0,75} (NRC, 1974), adjusted according to the growth phase and temperature of the environment (TABLE 3).

The experiments consisted of two digestibility tests, with the first test being performed with animals at the age of seven months using 12 dogs. The experimental period took eight days, comprising five days of adjustment and 5 days for collection of samples. During the whole period the animals stayed in individual partition receiving water as they wish and food under control, as previously described.

The second digestibility test was performed with 16 dogs, at the age of 15 months. This test took ten days, pursuant to recommendation of AAFCO (2000), being five days for adjustment and five for data collection. During the collection phase the animals were transferred for metabolic cages in order to facilitate the total collection of feces.

The feces were collected during five consecutive days, twice a day, weighed per animal, sampled and stored under refrigeration, so that by the end of the collection period it would be possible to form a composed sample per animal. The feces produced by the dogs during the experiment were assessed according to score presented on Table 4.

By the end of the experiment, the samples of feces were thawed and dried up in greenhouse at 55 ºC with forced ventilation for 48 hours. The food and feces samples were analyzed for determination of the dry matter contents (DM), crude protein (CP), crude fiber (CF), ether extract (EE), mineral matter (MM), Calcium (Ca) and phosphorus (P) in accordance with SILVA (1998). The fraction corresponding to the nitrogen-free extract (NFE) was set forth using the formula NFE = 100 - (%UM +%CP +%CF +%EE +%MM), being UM the humidity content of the sample. The metabolizable energy (ME) was calculated using the equation ME (Kcal/100g of food) = (3.5 x CP) + (8.5 x EE) + (3.5 x NNE).

It was used a completely randomized design, with 12 experimental units in the first test (6 repetitions per treatment), 16 experimental units in the second digestibility test (8 repetitions per treatment). The coefficients of apparent digestibility and metabolizable energy were initially submitted to the test of Bartlett in order to verify the homogeneity of the variances of the treatments, for further variance analysis and, the averages compared by the t test at 5% of probability.

TABLE 4 - SYSTEM OF EVALUATION OF THE CONSISTENCE OF FECES ACCORDING TO SCORES.

Score	Aspect of the feces
1	Liquid feces
2	Pasty feces
3	Soft pasty feces
4	Regular feces
5	Dry feces

Adapted from Case *et al*. (1998).

RESULTS AND DISCUSSION

The evaluation of the animals feces of the animals during the course of the experiment demonstrated that they were of good quality (score 4), with no occurrence of episodes of diarrhea or constipation.

As for the coefficients of apparent digestibility for dogs at the age between seven and 15 months, significant differences were not observed between the control and CLA groups (Tables 5 and 6). Such data corroborate those found by COUSINS *et al*. (2004).

COUSINS *et al*. (2004) conducted an experiment of digestibility test with adult Beagle dogs, using diets with and without CLA (0.5%) and they observed that there was not significant difference among the coefficients of digestibility for dry matter, energy, protein, fat and nitrogen-free extract between the control and CLA diets.

However, significant difference was observed for metabolizable energy between the control and CLA diets, both for the digestibility test with dogs at the age of seven months and 15 months, where the CLA group displayed greater metabolizable energy than the control group. TERPSTRA *et al*. (2002) observed that the CLA increased the excretion of energy in the feces and there was a significant decrease in the apparent digestibility of raw energy, as well as, the apparent digestibility of fat in mice that received restricted feeding with CLA was significantly smaller than the control group, contradicting the results obtained in

TABLE 5 - COEFFICIENTS OF DIGESTIBILITY AND METABOLIZABLE ENERGY OF DIETS SUPPLIED TO DOGS AT THE AGE OF SEVEN MONTHS.

Diet	Coefficient of Digestibility (%)				ME
	DM	CP	EE	NFE	(kcal/kg)
Control	92.11±1.97	84.03±3.23	90.43±2.97	91.28±2.01	3385.2^{b} ± 111.2
CLA	92.65±0.63	84.04±1.95	92.34±1.03	91.10±0.83	3547.6^{a}± 51.6
P	0.5428	0.9933	0.1686	0.8476	0.0088
CV (%)	3.61	3.02	2.56	1.60	3.42

Control = Diet with no inclusion of CLA; CLA = Inclusion of 1% of CLA to the diet (% DM).
DM = Dry matter; CP = Crude protein; EE = ether extract; NFE = nitrogen-free extract; ME = Metabolizable energy.
^{a,b} = Averages followed by different small letters at the column are differ among themselves at 5% of significance according to t Test.
P = Probability Value.
CV = Coefficient of variation.

TABLE 6 - COEFFICIENTS OF DIGESTIBILITY AND METABOLIZABLE ENERGY OF DIETS SUPPLIED TO DOGS AT THE AGE OF 15 MONTHS.

Diet	Coefficient of Digestibility (%)				ME
	DM	CP	EE	NFE	(kcal/kg)
Control	92.37±0.45	85.09±1.12	93.97±1.21	91.22±1.25	3472.9[b] ± 36.6
CLA	92.86±1.18	86.50±2.53	93.99±0.97	92.59±1.46	3581.7[a]± 63.5
P	0.2941	0.1739	0.9661	0.0644	0.0009
CV (%)	0.97	2.36	1.13	1.62	2.13

Control = Diet with no inclusion of CLA; CLA = Inclusion of 1% of CLA to the diet (% DM).
[a,b] = Averages followed by different small letters at the column are differ among themselves at 5% of significance according to t Test.
DM = Dry matter; CP = Crude protein; EE = ether extract; NFE = nitrogen-free extract;
ME = Metabolizable energy.
P = Probability Value.
CV = Coefficient of variation.

The age of the dog is an important characteristic that should be considered when construing the results of nutritional experiments and clinical cases (SWANSON *et al*., 2004). The authors state that the smaller digestibility of nutrients observed in young animals may be due to smaller absorptive capacity, smaller production of pancreatic and intestinal enzymes or differences in the bacterial population in the large intestine. Young dogs present small intestine of larger size, weight and surface area, when adjusted to live weight, compared to the adult animals. The absorption surface, however, becomes larger in adult dogs in function of the largest body size (PAULSEN *et al*., 2003). Possibly because of the little difference of age among the animals assessed, no expressive differences were found when comparing digestibility values among dogs at the age between seven and 15 months.

The results found allow to infer that the use of CLA in dog food may be an important tool in the nutrition of dogs, taking into account the several benefits presented in the literature concerning the metabolic aspects (BISSONAUTH *et al*., 2006), absence of effect on the digestibility of nutrients and better use of the energy contained in the food, evidenced by this work, for dogs in the growth phase and adult.

In studies performed with mice, WEST *et al*. (1998) found out a decrease of the energy intake and fat store, besides the increase of the metabolic speed and breathing rate in animals that received diet with CLA. Such effects result in decrease of the build-up of body fat (OHNUKI *et al*., 2001; TERPSTRA *et al*., 2002).

It is recommended that future researches with more evaluations besides the digestibility test are performed, as measurement of the consumption of oxygen, to verify possible alteration in the metabolic rate, and use of objective methods to verify alteration of body composition.

CONCLUSIONS

The coefficients of apparent digestibility of nutrients and dry matter were not affected by the inclusion of CLA in diets for dogs of Beagle type at the age between seven and 15 months.

The metabolizable energy of the food supplemented with CLA showed to be significantly superior to the control food in both digestibility tests.

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