THE EFFECT OF L-CARNITINE ON DIGESTIBILITY AND CARNITINE METABOLISM IN PIGEONS (*COLUMBA LIVIA DOMESTICA*) FED CORN OR PEAS

Het effect van L-carnitine op verteerbaarheid en carnitinemetabolisme bij duiven (Columba livia domestica) gevoed met maïs of erwten

G.P.J. Janssens[1], A.M. Abd-Ellah[2], H. De Rycke[1], M. Hesta[1], R.O.M. De Wilde[1]

[1]Laboratory of Animal Nutrition, Faculty of Veterinary Medicine, Ghent University, Heidestraat 19, B-9820 Merelbeke, Belgium
[2]Department of Animal Hygiene, Faculty of Veterinary Medicine, Assiut University, Egypt
geert.janssens@UGent.be

ABSTRACT

Sixteen adult female pigeons were randomly divided into two groups that were fed either corn or peas. Within each group, four pigeons received a supplement of 2.5 g L-carnitine/l drinking water. After an adaptation period of 7d, all excreta were gathered per bird in a 7d collection period. Coefficients of apparent digestibility of dry matter, organic matter, crude fat, crude fiber and nitrogen-free extract and apparent nitrogen retention were calculated from proximate analyses of feedstuffs and excreta. Blood samples were taken at the end of the trial and plasma samples were analyzed for carnitine and related compounds. Supplementation of L-carnitine did not affect digestibility but it did reduce apparent nitrogen retention in the corn-fed pigeons. Plasma analysis of γ-butyrobetain gave indications for a negative feedback mechanism of high L-carnitine dosage on *de novo* L-carnitine synthesis.

SAMENVATTING

Zestien volwassen vrouwelijke duiven werden at random verdeeld in twee groepen die ofwel met maïs, ofwel met erwten werden gevoed. Binnen elke groep kregen vier duiven een drinkwatersupplement van 2,5 g L-carnitine/l. Na een adaptatieperiode van 7 dagen werd in een 7 dagen durende collectieperiode al de mest per vogel verzameld. De coëfficiënten van de schijnbare verteerbaarheid van droge stof, organische stof, ruw vet, ruwe vezel en overige koolhydraten en van de schijnbare stikstofretentie werden berekend uit de Weendeanalyse van de voedermiddelen en de excreta. Op het einde van de proef werden bloedstalen genomen en de plasmastalen werden geanalyseerd voor carnitine en verwante verbindingen. De supplementatie van L-carnitine had geen invloed op de verteerbaarheid maar verminderde de schijnbare stikstofretentie bij de met maïs gevoederde duiven. De analyse van γ-butyrobetaïne in plasma laat een negatief feedbacksysteem vermoeden van hoge L-carnitinesupplementatie op de *de novo* synthese van L-carnitine.

INTRODUCTION

Fatty acid metabolism is important in pigeons because of the need for fat-derived energy during flight (Parker and George, 1975) and the production of the fat-rich crop-milk during breeding (De Smeth *et al.*, 1981). The vitamin-like substance L-carnitine is an intermediary factor in fatty acid metabolism because it transports acyl compounds through the inner mitochondrial membrane (Fritz, 1955). Previous studies in pigeons have shown that L-carnitine supplementation of typical pigeon feeds improves energy utilization efficiency in terms of growth (Janssens *et al.*, 2001), reproduction (Janssens *et al.*, 2000a), activity (Janssens *et al.*, 1998) and immune response (Janssens *et al.*, 2000b). The fact that L-carnitine supplementation affects this wide range of physiological aspects, indicates that its function at the cellular level is probably similar in all tissues. The intestinal mucosa is rich in L-carnitine (Flores *et al.*, 1996), which gives eviden-

ce for its importance in the proper functioning of mucosal cells.

Lecluyse *et al.* (1993) found that the mucosal absorption of certain drugs was stimulated by the provision of long-chain acyl carnitines. This suggests that mucosal absorption is subject to the L-carnitine status of the mucosal cells. Nevertheless, information on the effect of carnitine on nutrient absorption is hard to find. The present study was intended as an initial attempt to investigate the potential effect of L-carnitine supplementation on digestibility in pigeons fed two different feedstuffs: peas, as an example of a protein-rich feedstuff, and corn, as an example of a carbohydrate-rich feedstuff. In an attempt to clarify potential effects, carnitine-related metabolites are determined in the blood plasma: γ-butyrobetain is the last step in the biosynthesis of L-carnitine (Fritz, 1955) and can thus provide information on the de novo synthesis in the body, acetylcarnitine indicates the level of acylation of L-carnitine and isobutyrylcarnitine can give indications on the catabolism of protein, as it is a metabolite of valine (Roe *et al.*, 1998).

MATERIAL AND METHODS

Animals and housing

Sixteen adult female pigeons (*Columba livia domestica*) were housed in group cages measuring 2.2 m high, 1.5 m wide and 2.2 m long.

All birds were fed a commercial mixture of grains (Moulding Extra, Versele-Laga Ltd., Deinze, Belgium) *ad libitum* for two months. After this period, the pigeons were individually housed for fifteen days in battery cages on wired floor, measuring 25 cm high, 40 cm wide and 80 cm long.

They had *ad libitum* access to fresh drinking water. A solid mineral supplement and a mixture of ground shells and stones were available *ad libitum*. Although these supplements made the interpretation of the results more complex, they were thought to be necessary to supply minerals and to maintain the proper functioning of the gizzard and crop.

Treatments

Eight pigeons (group ZM) received a diet consisting of 25 g corn grains (*Zea mays.*) per day, whereas the other eight pigeons (group PS) received a diet of 25 g peas (*Pisum sativum L.*) per day. The nutrient composition of the corn grains and peas is presented in Table 1.

Table 1. Chemical analysis of corn and peas.

	Corn	Peas
Dry matter (g/kg)	864.2	871.0
Crude ash (g/kg)	13.9	33.5
Crude protein (g/kg)	90.6	207.1
Ether extract (g/kg)	51.3	22.6
Crude fiber (g/kg)	28.5	50.3
Nitrogen-free extract (g/kg)	679.9	557.5
Organic matter (g/kg)	850.3	837.5

Within each group, four pigeons (CAR) were randomly assigned to receive a drinking water supplement of 2.5 g L-carnitine per liter (Lonzagroup Ltd., Basel, Switzerland). The other four pigeons (CON) in each group received unsupplemented drinking water.

A 7d-adaptation period was followed by a 7d-collection period during which a daily total collection of the excreta was performed for each pigeon. All pigeons were weighed individually at d1, d8 and d15. Daily intakes of feed, water, pickstone and grit were registered both for the preliminary period and for the collection period.

Blood samples were taken from a leg vein of each pigeon at the end of the collection period in heparinized tubes. The plasma was stored at –20°C until HPLC analysis.

Chemical analyses

Corn, peas and excreta samples were subjected to proximate analysis.

Carnitine and related compounds were analyzed by HPLC as described by Janssens *et al.* (1999).

The carnitine content of the corn and peas was not determined because vegetal feedstuffs contain only negligible amounts of L-carnitine when compared to the applied dose (Baumgartner and Blum, 1997).

Statistical analysis

All data were statistically analyzed using a two-way analysis of variance with L-carnitine dose and diet as independent variables. In the event of an over-all significant effect, the differences between the separate groups were evaluated using a Duncan post-hoc test (Neter *et al.*, 1991).

Table 2. Nutrient digestibility and nitrogen retention in pigeons with or without L-carnitine supplementation fed corn of peas (mean ± SD).

	Peas		Corn	
	CON	**CAR**	**CON**	**CAR**
AD dry matter (%)	63.3±2.9 [a]	65.9±2.3 [a]	84.4±0.9 [b]	83.1±1.1 [b]
AD crude fat (%)	76.8±1.7 [a]	76.9±3.4 [a]	84.5±3.9 [b]	84.1±2.5 [b]
AD crude fiber (%)	14.2±6.9 [a]	22.0±3.1 [a]	17.9±4.5 [a]	12.9±6.5 [a]
AD nitrogen-free extract (%)	85.4±1.0 [a]	85.1±0.9 [a]	92.2±0.1 [b]	92.4±1.0 [b]
AD organic matter (%)	67.9±1.3 [a]	68.4±2.3 [a]	86.3±0.6 [b]	84.8±0.9 [b]
Nitrogen retention (%)	37.2±1.7 [a]	38.1±6.7 [a]	61.1±2.2 [b]	47.6±4.8 [c]

AD: apparent digestibility
CAR: 2.5 mg L-carnitine/l drinking water; CON: no supplement.
[a,b] Different subscripts indicate significant differences within a row ($P < 0.05$).

RESULTS

Body weight

The initial body weights were not significantly different between the groups. The average initial weight was 427 ± 32 g. During the preliminary period, the average body weight decreased to 403 ± 38 g ($P < 0.001$). Body weight did not change during the collection period: the average final weight was 401 ± 30g.

Water intake

No significant differences were seen in water intake due to supplementation or feed. The water intake of the pigeons averaged 34 ± 8 ml/d. This implies that the daily L-carnitine intake for the L-carnitine supplemented pigeons was on average 85 ± 20 mg/pigeon.

Apparent digestibility and nitrogen retention

Table 2 shows the apparent digestibility coefficients and the apparent nitrogen retention coefficients of each of the four groups. No significant differences were seen due to L-carnitine supplementation except for a significantly lower apparent level of nitrogen retention in the corn-fed group.

The coefficients of apparent nitrogen retention and apparent digestibility of dry matter, organic matter, ether extract and nitrogen-free extract were all significantly higher with corn than with peas ($P < 0.001$). The apparent digestibility coefficient of crude fiber was not significantly different.

Plasma analysis

The results of the HPLC analyses are presented in Table 3. Non-esterified carnitine levels were significantly higher in the corn-fed pigeons than in the peas fed pigeons ($P = 0.033$), although the post-hoc was unable to indicate the differences. The acetylcarnitine and isobutyrylcarnitine levels were not influenced by the treatments, but γ-butyrobetain was significantly higher in corn-fed pigeons without L-carnitine supplementation than in the other three groups ($P = 0.042$).

DISCUSSION

Coefficients of apparent nutrient digestibilities and apparent nitrogen retention for corn and peas were similar to those in other digestibility trials with pigeons (Hullar *et al.*, 1999; Sales and Janssens, 2003b). In both cases, several coefficients differed substantially from those obtained in common poultry, as described by Hullar *et al.* (1999). The reasons why these figures can differ between chickens and other birds are discussed by Sales and Janssens (2003a).

The present trial provided no evidence for the hypothesis that L-carnitine supplementation could enhance nutrient absorption by mucosal cells: the only coefficient for which an effect of L-carnitine supplementation was demonstrated is apparent nitrogen re-

Table 3. Plasma levels (nmol/ml) of carnitine, acylcarnitines and γ-butyrobetain in pigeons with or without L-carnitine supplementation fed corn or peas (mean ± SD; N = 4).

	Corn		Peas	
	CON	**CAR**	**CON**	**CAR**
Non-esterified carnitine	107 ± 65 [a]	111 ± 93 [a]	29 ± 5 [a]	42 ± 17 [a]
Acetylcarnitine	49 ± 29 [a]	47 ± 44 [a]	31 ± 7 [a]	22 ± 12 [a]
Isobutyrylcarnitine	137 ± 35 [a]	135 ± 72 [a]	125 ± 93 [a]	138 ± 88 [a]
γ-Butyrobetain	375 ± 270 [b]	75 ± 23 [a]	109 ± 50 [a]	31 ± 22 [a]

CAR: 2.5 mg L-carnitine/l drinking water; CON: no supplement.
[a,b] Different subscripts indicate significant differences within a row ($P < 0.05$).

tention for corn. It cannot be excluded that this effect was only at the level of protein metabolism after absorption. As urinary nitrogen and fecal nitrogen were not separated, an effect on protein digestibility could not be demonstrated.

Studies have demonstrated that L-carnitine supplementation can improve nitrogen retention (e.g. pigs: Heo *et al.*, 2000). The fact that it also occurs in parenteral nutrition (Böhles *et al.*, 1984) indicates an effect post-absorption. The present trial revealed decreased apparent nitrogen retention due to L-carnitine supplementation in corn-fed pigeons instead. To explain these contrasting findings, one could suggest that the fairly high dosage in the present study may have exerted a negative feedback mechanism. Plasma analysis did indeed show a reduced level of the L-carnitine precursor γ-butyrobetain in the L-carnitine supplemented pigeons (in the corn group), suggesting that *de novo* L-carnitine synthesis was hampered. The first evidence for such a mechanism was described by Janssens *et al.* (2000a).

As the primary function of L-carnitine is to support fatty acid combustion in the mitochondria (Fritz, 1955), the need for it will increase with increasing dietary fat intake, which coincides with the fact that the highest level of γ-butyrobetain was in the unsupplemented corn-fed pigeons. If the high dosage of L-carnitine impaired optimal fatty acid combustion, it is obvious that other energy sources, such as amino acids, came into play and, as a consequence, nitrogen retention dropped. This was not seen in the pigeons fed on peas because the fat level in peas is far lower than in corn, especially when expressed in terms of weight per gram protein.

The plasma level of the (non-esterified) carnitine itself was not altered by L-carnitine supplementation, but feeding peas induced a higher plasma non-esterified carnitine level in comparison to feeding corn. Corn contains more fat than peas, which can explain the greater flow of free carnitine from blood towards tissues, in order to support the transport of acyl groups to the mitochondria. The plasma concentration of acetylcarnitine might reflect the level of carnitine acylation in the mitochondria, though this could not be demonstrated.

Isobutyrylcarnitine is an intermediary product of the catabolism of valine, a branched-chain amino acid (Roe *et al.*, 1998). A change in this compound can thus give an idea of the use of amino acids for energy production. The plasma levels in this trial provided no evidence for a different use of protein for energy generation, although the ratio between protein and the other energy substrates, fat and carbohydrates, is quite higher in peas than in corn. A plausible explanation is that even the low protein:energy ratio in corn is sufficient to cope with the protein demands of adult, non-productive pigeons.

To conclude, the present study could not prove an effect of L-carnitine supplementation on digestibility, though nitrogen retention was altered, presumably by a negative feedback mechanism. Future studies should investigate this topic at lower dosages of L-carnitine.

ACKNOWLEDGEMENTS

We want to thank the technical staff of the Laboratory of Animal Nutrition. We also want to acknowledge

Lonzagroup and Versele-Laga for kindly supplying L-carnitine and the feedstuffs, respectively.

LITERATURE

Baumgartner M., Blum R. (1997). Typical L-carnitine contents in feedstuffs. *Folder Lonzagroup Ltd*, Basel, Switzerland.

Böhles H., Segerer H., Fekl W. (1984). Improved N-retention during L-carnitine-supplemented total parenteral nutrition. *Journal of Parenteral and Enteral Nutrition 8*, 9-13.

Desmeth M., Messeyne M., Schuermans G., Vandeputte-Poma J., Vandergeynst F. (1981). Effect of age and diet on the fatty acid composition of triglycerides and phospholipids from liver, adipose tissue and crop of the pigeon. *Journal of Nutrition 111*, 1480-1488.

Flores C.A., Hu C., Edmond J., Koldovsky O. (1996). Milk carnitine affects organ carnitine concentration in newborn rats. *Journal of Nutrition 126*, 1673-1682.

Fritz I.R. (1955). The effects of muscle extracts on the oxidation of palmitic acid by liver slices and homogenates. *Acta Physiologica Scandinavica 34*, 367-385.

Heo K., Odle J., Han I.K., Cho W., Seo S., van Heugten E., Pilkington D.H. (2000). Dietary L-carnitine improves nitrogen utilization in growing pigs fed low-energy, fat-containing diets. *Journal of Nutrition 130*, 1809-1814.

Hullar I., Meleg I., Fekete S., Romvari R. (1999). Studies on the energy content of pigeon feeds. I. Determination of digestibility and metabolizable energy content. *Poultry Science 78*, 1757-1762.

Janssens G.P.J., Buyse J., Seynaeve M., Decuypere E., De Wilde R. (1998). The reduction of heat production in exercising pigeons after L-carnitine supplementation. *Poultry Science 77*, 578-584.

Janssens G.P.J., De Rycke H., Hesta M., De Wilde R.O.M. (1999). Analysis of carnitine, betain, γ-butyrobetain, and separate short-chain acylcarnitines in pigeon plasma, crop milk and tissues by HPLC coupled with photometric detection. *Biotechnology Techniques 4*, 231-234.

Janssens G.P.J., Hesta M., Debal V., Debraekeleer J., De Wilde R.O.M. (2000a). L-carnitine supplementation in breeding pigeons: impact on zootechnical performance and carnitine metabolism. *Reproduction Nutrition Development 40*, 535-548.

Janssens G.P.J., Mast J., Goddeeris B.M., Cox E., Hesta M., De Wilde R.O.M. (2000b). Enhanced specific antibody response to bovine serum albumin in pigeons due to L-carnitine supplementation. *British Poultry Science 41*, 448-453.

Lecluyse E.L., Sutton S.C., Fix J.A. (1993). In vitro effects of long-chain acylcarnitines on the permeability, transepithelial electrical resistance and morphology of rat colonic mucosa. *Journal of Pharmacological and Experimental Therapeutics 265*, 955-962.

Parker G.H., George J.C. (1975). Effects of short and long-term exercise on intracellular glycogen and fat in pigeon pectoralis. *Japanese Journal of Physiology 25*, 175-84.

Roe C.R., Cederbaum S.D., Roe D.S., Mardach R., Galindo A., Sweetman L. (1998). Isolated isobutyryl-CoA dehydrogenase deficiency: an unrecognized defect in human valine metabolism. *Molecular Genetics and Metabolism 65*, 264-271.

Sales J., Janssens G.P.J. (2003a). Energy and protein nutrition of companion birds. *Vlaams Diergeneeskundig Tijdschrift 72*, 51-58.

Sales J., Janssens G.P.J. (2003b). Methods to determine metabolizable energy and digestibility of feed ingredients in the domestic pigeon (Columba livia domestica). *Poultry Science 82*, 1457-1461.