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Original article

L-carnitine supplementation in breeding pigeons: impact on zootechnical performance and carnitine metabolism

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Abstract — In the first experiment (Exp1), three consecutive breeding rounds were performed by two groups of six pigeon couples in order to study the impact of L-carnitine supplementation (80 mg·d^{-1}) of parent pigeons on zootechnical performance. Both in the second and third experiments (Exp2, Exp3), one breeding round was performed by two groups of six pigeon couples to reveal the biochemical background of the increase in squab growth, the limitation of body weight decrease in male parent birds and the tendency for an improved cumulative feed efficiency due to L-carnitine supplementation in Exp1. Growth improvement of the squabs with L-carnitine was only seen when the parent pigeons were supplemented, together with a marked rise in the body weight of the parent birds around hatching. Based on the results of the crop milk analysis, growth improvement was probably due to a quantitative impact on crop milk production. The crop milk from the supplemented groups in both Exp2 and Exp3 had increased levels of carnitine. Carnitine, γ-butyrobetaine and acetylcarnitine were increased in plasma samples of the supplemented parent pigeons. No differences were present in the squabs' plasma for these parameters. In the squabs of Exp3, no changes were seen in the proportional growth or the protein content of the heart, breast muscle and liver, but the breast muscle of the squabs from the supplemented group in Exp3 showed a considerable rise in carnitine and a marked decrease in γ-butyrobetaine.

carnitine / pigeon / growth / crop-milk / HPLC

Résumé — La supplémentation de L-carnitine chez les pigeons en couvaison : l'influence sur la performance zootechnique et des paramètres biochimiques. Dans la 1re expérience, six couples de pigeons ont produit trois couvées consécutives pour l'étude de l'effet de la supplémentation de L-carnitine (80 mg·j^{-1}) sur la performance zootechnique. Dans les 2e et 3e expériences, deux groupes de six couples de pigeons ont produit une couvée pour l'étude de l'action biochimique expliquant l'augmentation de la croissance des pigeonneaux, la limitation de la perte de poids chez les pigeons adultes mâles, et la tendance à l'amélioration de l'utilisation alimentaire cumulative due à la supplémentation en L-carnitine de l'expérience 1. Une amélioration de la croissance n'était constatée qu'en

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cas de supplémentation des pigeons adultes, accompagnée d'une augmentation remarquable du poids corporel des pigeons adultes au moment de l'éclosion des œufs. Vu les résultats des analyses du lait de pigeon, l'amélioration de la croissance semblait due à une influence quantitative sur la production du lait de pigeon. Le lait de pigeon du groupe supplémenté avait un taux élevé de carnitine dans les deux expériences. La carnitine, la γ-butyrobétaïne et l'acétylcarnitine étaient augmentées dans les échantillons de sang des pigeons adultes supplémentés. Il n'y avait aucune différence dans les échantillons de sang des pigeonneaux. Chez les pigeonneaux de l'expérience 3, il n'y avait aucune différence quant à la croissance proportionnelle ou le contenu protéinique du cœur, du muscle pectoral et du foie, mais le muscle pectoral des pigeonneaux du groupe supplémenté de l'expérience 3 contenait plus de carnitine et moins de γ-butyrobétaïne.

carnitine / pigeon / croissance / lait de pigeon / HPLC

1. INTRODUCTION

The growth performance of pigeon (*Columba livia domestica*) squabs is of crucial importance for the production of pigeon meat. The development of squabs is also considered as one of the determining factors for flight performance in racing pigeons. Moreover, changes in the condition of racing pigeons during breeding will have a considerable impact on their flight performance.

Lipids more than other nutrients, play an essential role in the nutrition of the pigeon squab. In most newly-hatched birds, the residual yolk-sac serves as an extra nutritional source [11]. Pigeon yolk consists of 27.1% lipids on the 11th day of incubation and of 12.0% lipids around hatching [27]. Furthermore, squabs are exclusively fed by the crop milk of both parents during the first days after hatching. Because crop milk has a fat content on dry matter basis of 33.8% immediately after hatching decreasing to 16.5% on the 19th day after hatching [8], the animals continue to receive a fat-rich nutrition for several days post-hatching.

From the above data it is clear that lipid metabolism has a major role in the breeding of pigeons. It has been known for a long time that L-carnitine has a determining function in the supply of fatty acids to the mitochondria for energy production [9]. Therefore, this study was aimed at investigating the effect of L-carnitine supplementation in breeding pigeon or squabs on farming performance and carnitine metabolism.

2. MATERIALS AND METHODS

2.1. Experiment 1 (Exp1)

2.1.1. Animals, housing and nutrition

In each of two cages, six male and six female pigeons *(Columba livia domestica)* were allowed to form couples on a random basis. The cages were 1.5 m long, 2 m high and 2 m wide on a wired floor. Water and a solid mineral supplement (Pickstone, Versele-Laga Ltd., Deinze, Belgium) were available ad libitum per cage. Each breeding pair had a separate nest box with devices for feed and a mixture of ground shells and stones (Versele-Laga Ltd., Deinze, Belgium). The feed consisted of a commercial mixture of whole grains and seeds (Breeding Extra, Versele-Laga Ltd., Deinze, Belgium). The composition of the grain mixture is presented in Table I. The L-carnitine content was not determined since most vegetable feedstuffs are very low in L-carnitine, for instance 5–10 $mg \cdot kg^{-1}$ in corn [15]. From previous studies it was known that the maximal feed intake per pigeon would not exceed 100 $g \cdot day^{-1}$. Hence, the error by not taking into account the L-carnitine content in the feed was negligible compared to the L-carnitine supplement mentioned further on.

Supplying about only 110% of the estimated ad libitum intake restricted selection of feed ingredients by the pigeons.

Table I. Composition of the mixture of whole grains and seeds.

Chemical composition	Exp1 g·kg^{-1}	Exp2 and 3 g·kg^{-1}
Moisture	121	115
Crude ash	20	19
Crude protein	136	143
Ether extract	47	36
Crude fiber	55	47
Crude starch	523	530
Sugars	24	28
Nitrogen-free extract	621	640
Apparent metabolisable energy for poultry (MJ·kg^{-1})	12.7	12.7
Feedstuff composition	g·kg^{-1}	g·kg^{-1}
Corn (*Zea mays*)	350	365
Millet (*Setararia italica*)	50	0
Milocorn (*Sorghum caffrorum*)	0	80
Pea (*Pisum sativum*)	350	320
Safflowerseed (*Carthamus tinctorius*)	30	20
Vetch (*Vicia sativa*)	0	15
Wheat (*Triticum aestivum*)	220	200

2.1.2. Treatments

From 5 days before until 5 days after hatching of the eggs, both male and female pigeons in the first cage (CAR) received a daily oral dose of 80 mg L-carnitine in 0.5 mL of distilled water. This was accomplished by intubation with a 1 mL syringe without a needle. The parents in the second cage (CON) were administered a placebo solution of 0.5 mL of distilled water in the same way. Three consecutive breeding rounds were performed by each breeding pair to study the possible interaction of the duration of breeding with L-carnitine administration. The squabs were weaned 24 days after hatching.

2.1.3. Measurements

The parent birds were weighed twice a week during the experiment. The squabs were weighed daily from hatching to weaning. Nutrient intakes were also measured daily.

A daily notation indicating which of the two parents was on the nest was made. Minimum and maximum environmental temperatures were also recorded daily.

The grain mixture was subject to proximate analysis. The apparent metabolisable energy content was calculated with the formula for poultry of the European Union:

$$AMEp = 0.155\ CP + 0.343\ EE + 0.167\ St + 0.130\ Su$$

where AMEp = apparent metabolisable energy (MJ·kg^{-1} feed), CP is g of crude protein, EE is g of ether-extract, St is g of starch and Su is g of sugars (mono- and disaccharides) per kg of feed [13].

2.2. Experiment 2 (Exp2)

Animals, housing and nutrition were similar to those in Exp1. The composition of

the grains and seeds mixture is also given in Table I.

From the hatching day on, the squabs in one group (CAR) received an oral supplement of L-carnitine: by means of a syringe without a needle, 40 mg L-carnitine, dissolved in 0.5 mL water, was intubated per squab each day. The squabs in the other group (CON) were given 0.5 mL water as a placebo solution in the same way. The squabs were weighed and intubated every morning (about 09:00) from hatching to weaning at 24 days of age. Samples of crop milk were taken in the afternoon (about 15:00) from the living squabs at 5 days of age (d5). The procedure is described by Janssens et al. [10]. The crop milk was taken from the squabs because studies have shown that the composition of crop milk taken from squabs is more constant than when taken from parent pigeons [23]. Blood samples were drawn from a leg vein of the squabs at weaning. No blood samples were taken at d5 because at that age, this would have retarded the squabs' growth. Blood plasma and crop milk samples were stored at –20 °C until analysis.

Crop milk and plasma samples were analysed for carnitine, γ-butyrobetaine and acylcarnitine content according to Janssens et al. [10].

2.3. Experiment 3 (Exp3)

Animals, housing and nutrition were similar to those in Exp2.

The parent pigeons were supplemented with 80 mg L-carnitine per day for only 5 days before hatching until 5 days after hatching of the first egg.

The squabs were weighed daily and the parent pigeons were weighed after oviposition of the first egg (d–19), at hatching of the first egg (d1) and at weaning (d24). At d5, blood plasma samples were taken from the parent pigeons and crop milk samples from the squabs. At weaning age, the squabs were euthanised. Their liver, heart and breast muscle were dissected and weighed and blood plasma samples were taken from the aorta. All samples were stored at –20 °C until analysis.

The liver, heart, breast muscle, crop milk and plasma were analysed for carnitine, γ-butyrobetaine and acylcarnitine content according to Janssens et al. [10].

The protein contents of the liver, heart, breast muscle and crop milk were determined with the Dumas technique [12].

2.4. Data analysis

Cumulative feed utilisation efficiency for growth was calculated per nest by dividing the weight gain of both squabs from hatching by the cumulative feed intake of both parent pigeons and squabs together. Body weight evolution of the parent pigeons was not taken into account for this parameter. To analyse the body weight of the squabs, the body weight of the parent pigeons, the daily feed intake and the cumulative feed utilisation efficiency, a mixed model repeated measures analysis was applied according to Littell et al. [14], using the MIXED procedure in SAS 6.12 (Statistical Analysis Systems Inc., Cary, USA). Initially, the Schwarz-Bayesian goodness-of-fit Criterion (SBC) was applied to detect the best fitting covariance structure. Then, fixed effects were tested and the means with their standard errors were calculated using the LSMEANS statement and compared using the ESTIMATE statement of PROC MIXED.

Initial body weight differences were tested by variance analysis and the incubation behaviour data were tested by chi-square analysis. Correlations between the intake of water and mineral supplements and the environmental temperature were estimated by Pearson correlation coefficients.

Changes in the parents' body weight were analysed by the repeated measures technique [18].

In all other cases, general linear model variance analysis was applied [18]. Differences between groups were determined by Scheffé tests.

All statistical analyses, including the calculation of means and standard errors and deviations, were based on Neter et al. [18] and were performed with SPSS 7.5 (SPSS Inc., Chicago, USA), except for the mixed model repeated measures that were performed by SAS 6.12 (SAS Inc., Cary, USA).

3. RESULTS

3.1. Experiment 1

No significant differences were found between the three breeding rounds. Therefore, all data were pooled over the breeding rounds.

3.1.1. Growth of the squabs

The Schwarz Bayesian criterium (SBC) for the squab weight data was found to be highest for the compound symmetry model. A highly significant interaction was present between squab age and the L-carnitine treatment ($p < 0.001$). The average growth pattern of the squabs in the two treatment groups is presented in Figure 1 and shows that the squab weight is significantly different at several days in the development. Towards the end, the difference disappears.

3.1.2. Nutrient intake

The fact that the water consumption was primarily determined by the environmental temperature is indicated by the correlation coefficient of 0.59 ($p < 0.001$; $N = 93$) between these two variables. The intake pattern of mineral supplements was also correlated with both water intake (0.52; $p < 0.001$; $N = 100$) and environmental temperature (0.38; $p < 0.001$; $N = 122$). L-Carnitine did not induce changes in the consumption of water and mineral supplements.

Figure 2 shows the profile of the daily feed intake related to squab age. The SBC for the feed intake data was highest for the compound symmetry model. No evidence was found for differences between the treatments. The increase in feed intake slowed down from about 17 days of age and coincided with high standard errors.

3.1.3. Cumulative feed utilisation efficiency

Based on the SBC values for the cumulative feed utilisation efficiency data, the

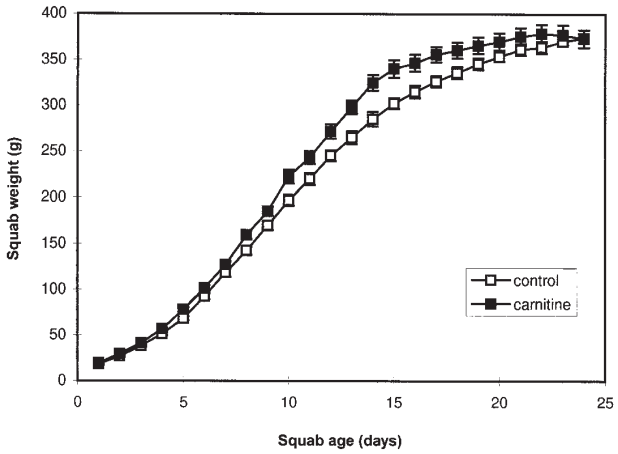

Figure 1. The growth pattern per squab of six nests with two squabs each with or without L-carnitine supplementation of the parent pigeons. Error bars represent standard error values ($N = 36$). No significant differences were found between the three breeding rounds. All data were pooled over the breeding rounds.

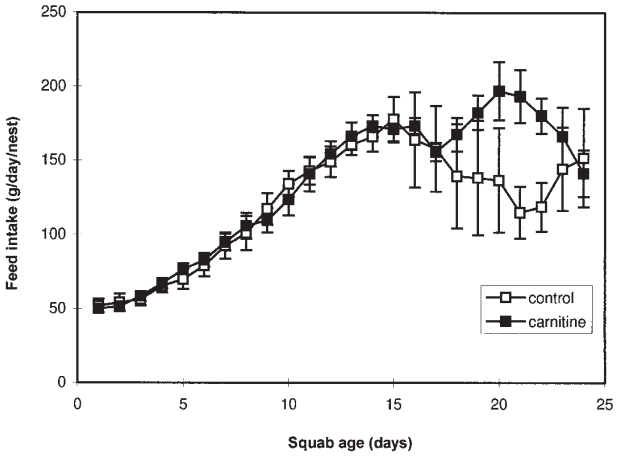

Figure 2. The daily feed intake per two-squab nest with or without L-carnitine supplementation of the parent pigeons. Error bars represent standard error values (N = 18). No significant differences were found between the three breeding rounds. All data were pooled over the breeding rounds.

compound symmetry structure fitted best for the covariance structure. In general, the cumulative feed utilisation efficiency increased rapidly to d9 and then decreased gradually to weaning age (d24). The cumulative feed utilisation efficiency was systematically improved by L-carnitine supplementation (Fig. 3), although the differences did not reach the level of significance (p = 0.066). This efficiency effect was larger when the lesser weight loss in the L-carnitine supplemented male parent pigeons was taken into account.

3.1.4. Weight loss of the parent pigeons (Tab. II)

For the body weight data of the parent pigeons, a simple covariance structure was used. The initial body weights of the male parent pigeons were higher than those of the female pigeons ($p < 0.001$). There were no significant differences in initial body weights between the treatments. All parent pigeons lost weight during the experiment (p = 0.007), but in the male pigeons these

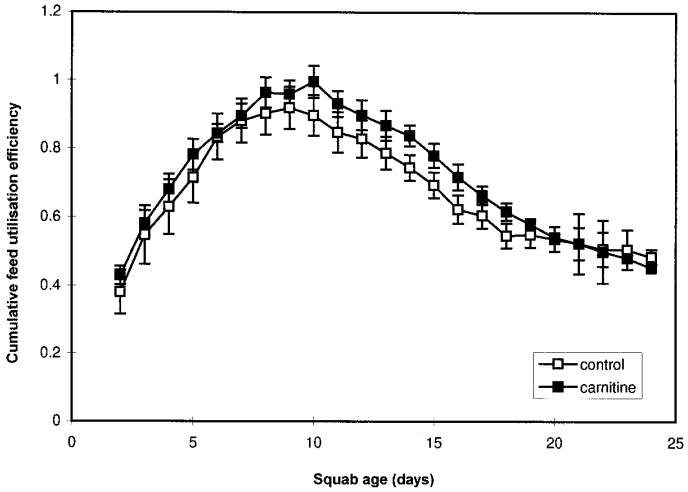

Figure 3. The cumulative feed efficiency for the two-squab nests, with or without L-carnitine supplementation of the parent pigeons, was calculated by dividing the squabs' weight gains from hatching by the cumulative feed intake of both parent pigeons and squabs from hatching per nest. Error bars represent standard error values (N = 18). No significant differences were found between the three breeding rounds. All data were pooled over the breeding rounds.

Table II. Body weights of the parent pigeons with or without L-carnitine supplementation (n = 24).

	Female		Male		SEM
	CAR	CON	CAR	CON	
	N = 6				
Initial weight (g)	506[a]	496[a]	553[b]	555[b]	8
Average weight during breeding (g)*	467[a]	457[a]	519[b]	501[c]	7
Relative weight loss (% of initial weight)*	6[ab]	6[ab]	5[a]	8[b]	0.8

* Over the three breeding rounds.
CAR = carnitine supplemented group; CON: control group.
[a,b,c] Different superscripts within a row indicate significant differences ($p < 0.05$).

losses were limited by L-carnitine supplementation, as indicated by the relative body weight loss interaction between sex and treatment ($p = 0.009$).

3.1.5. Incubation behaviour

During morning recordings, 71% of the female pigeons were breeding versus 25% males and 4% empty nests. For afternoon observations, 56% of the male pigeons were on the nest versus 27% females and 17% empty nests. Chi-square analysis showed these breeding patterns to be highly significant ($p < 0.001$). No effects of L-carnitine were detected.

3.2. Experiment 2

3.2.1. Growth of the squabs

Based on Schwarz Bayesian Criteria (SBC) analysis, the compound symmetry model was found to be the most appropriate for the covariance structure of the squab weight data. No significant treatment effect on growth could be detected; the growth

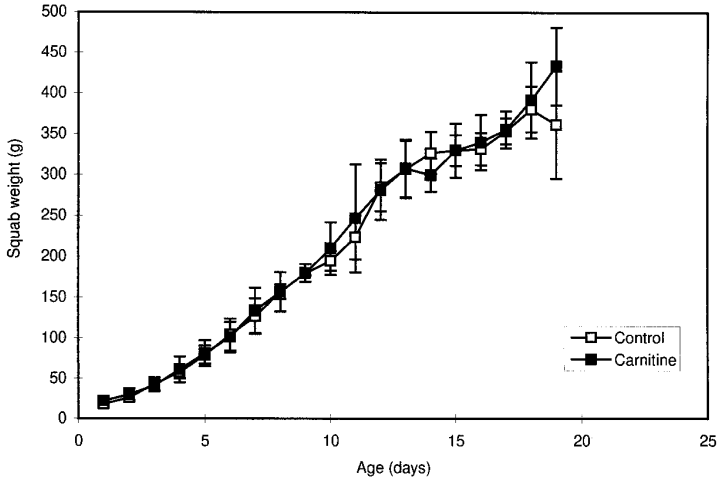

Figure 4. Growth profile of the squabs in Exp1 (mean ± standard error; *N* for control = 8; *N* for carnitine = 12).

Table III. Concentrations of carnitine, γ-butyrobetaine and acetylcarnitine in the crop milk in Exp1 (n = 10).

	CON	CAR	P-value	SEM
Carnitine, nmol·mL^{-1}	217	9 054	0.023	2 096
γ-Butyrobetaine, nmol·mL^{-1}	3 128	5 473	NS	833
Acetylcarnitine, nmol·mL^{-1}	195	194	NS	52

CAR = carnitine supplemented group; CON: control group; NS = not significant at $p < 0.05$.

patterns of both groups of squabs were almost identical (Fig. 4). At no point could significant differences be identified.

3.2.2. Crop milk levels of carnitine, γ-butyrobetaine and acetylcarnitine

The individual variation was high (Tab. III). The crop milk samples from CAR had a significantly higher carnitine content than CON. Neither γ-butyrobetaine nor acetylcarnitine were influenced by L-carnitine supplementation.

3.3. Experiment 3

3.3.1. Growth of the squabs

The highest SBC value for the squab weight data was found for the unstructured model. The mixed model repeated measures only revealed a trend towards a treatment effect (p = 0.107), although Figure 5 shows that from hatching on, a gradual elevation of the body weight could be seen in the squabs from the L-carnitine supplemented pigeons compared to the control group. This trend disappeared again slowly towards d17.

3.3.2. Changes in body weight of the parent pigeons

The higher body weight of the male compared to the female pigeons was close to significance (p = 0.050), but the body weights at oviposition did not differ between the treatments (Tab. IV). However, at the time the squabs were newly-hatched, a very clear increase in body weight had occurred in the CAR treated group. In the CON group no weight increase was observed at that moment. At weaning, this weight difference was absent. No sex interactions with the L-carnitine treatment were observed.

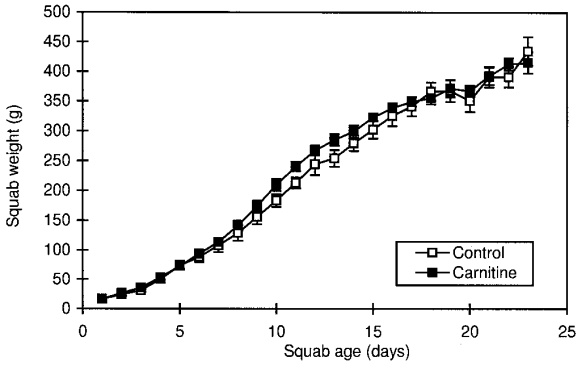

Figure 5. Growth profile of the squabs in Exp2 (mean ± standard error; N for control = 6; N for carnitine = 5).

Table IV. The body weights of the parent pigeons at oviposition (d-19), hatching (d1) and weaning (d24) in Exp2 (n = 24).

	Female		Male		SEM
	CAR	CON	CAR	CON	
	n = 6				
Oviposition	482^{aA}	482^{aA}	498^{aA}	523^{aA}	7
Hatching	480^{aA}	563^{bcB}	497^{abA}	593^{cB}	13
Weaning	470^{aA}	473^{aA}	487^{aA}	520^{aA}	8

^{a,b,c} Different superscripts within a row indicate significant differences ($p < 0.05$).
^{A,B} Different superscripts within a column indicate significant differences ($p < 0.05$).
CAR = carnitine supplemented group; CON = control group; P-values are: 0.005 for treatment; 0.040 for sex; < 0.001 for time; not significant for treatment × sex; < 0.001 for treatment × time; not significant for sex × time and not significant for treatment × sex × time.

3.3.3. Proportional growth

Neither the absolute weight of the heart, the liver and the breast muscle, nor their weights related to the total body weight showed significant differences between the treatments (Tab. V).

3.3.4. Carnitine analyses

The plasma analysis of the parent pigeons showed no differences between the sexes. Therefore, the sex factor was left out of the final statistical analysis. The plasma carnitine and acetylcarnitine levels of the CAR parent pigeons were significantly higher than in the CON pigeons (Tab. VI). The γ-butyrobetaine level was not significantly influenced by the L-carnitine treatment, although a trend towards an L-carnitine induced increase in γ-butyrobetaine was noticed.

For the squabs, carnitine, γ-butyrobetaine and acetylcarnitine levels differed significantly between the different kinds of sample, i.e. heart, liver, breast muscle, crop milk and plasma (Tab. VII). For carnitine and γ-butyrobetaine an interaction with L-carnitine supplementation was found. Unsupplemented pigeons showed high carnitine and acetylcarnitine levels in breast muscle and liver whereas in the heart, only acetylcarnitine was high. It was notable that the livers from

Table V. Proportional growth measurements from the squabs in Exp2 (n = 12).

	CON	CAR	P-value	SEM
Total body weight, g	430	449	NS	51
Heart weight, g	5.3	5.4	NS	0.7
Heart weight, g·kg^{-1} body weight	12.4	12.2	NS	1.6
Liver weight, g	10.8	12.5	NS	2.2
Liver weight, g·kg^{-1} body weight	25.1	27.8	NS	3.7
Breast muscle weight, g	62	63	NS	6.7
Breast muscle weight, g·kg^{-1} body weight	145	141	NS	12.2

CAR = carnitine supplemented group; CON = control group; NS = not significant at $p < 0.05$.

Table VI. Carnitine, γ-butyrobetaine and acetylcarnitine content in the parent pigeons' plasma in Exp2 (n = 24).

	CON	CAR	*P*-value	SEM
Carnitine, nmol·mL^{-1}	132	477	< 0.001	49
γ-Butyrobetaine, nmol·mL^{-1}	1 653	2 456	(0.086)	234
Acetylcarnitine, nmol·mL^{-1}	92	157	0.028	15

[a,b,c] Different indices within a row indicate significant differences ($p < 0.05$).
CAR = carnitine supplemented group: CON = control group.

Table VII. Carnitine, γ-butyrobetaine and acetylcarnitine content in the squabs in Exp 2; *P*-values from the variance analysis on the carnitine, γ-butyrobetaine and acetylcarnitine levels in squabs of L-carnitine supplemented parent pigeons (n = 12).

Content in:	CON	CAR	*P*-value	SEM
		Carnitine		
Plasma, nmol·mL^{-1}	199	106	NS	37
Heart, nmol·g^{-1}	131	100	NS	84
Liver, nmol·g^{-1}	1 328	1 064	NS	346
Muscle, nmol·g^{-1}	1 392	8 141	(0.059)	1 820
Crop milk, nmol·mL^{-1}	59	1 522	0.019	335
		γ-Butyrobetaine		
Plasma, nmol·mL^{-1}	5 262	3 958	NS	681
Heart, nmol·g^{-1}	6 755	4 843	NS	688
Liver, nmol·g^{-1}	41 185	38 322	NS	3 910
Muscle, nmol·g^{-1}	19 528	1 147	<0.001	3 314
Crop milk, nmol·mL^{-1}	893	19	NS	296
		Acetylcarnitine		
Plasma, nmol·mL^{-1}	227	170	NS	27
Heart, nmol·g^{-1}	2 419	1 769	NS	496
Liver, nmol·g^{-1}	5 691	8 328	NS	846
Muscle, nmol·g^{-1}	2 185	1 066	NS	1 257
Crop milk, nmol·mL^{-1}	36	27	NS	8
P-values	Treatment	Tissue	Interaction	
Carnitine	0.002	0.001	0.003	
γ-Butyrobetaine	0.026	<0.001	0.013	
Acetylcarnitine	0.193	<0.001	0.346	

CAR = carnitine supplemented group; CON = control group; NS = not significant at $p < 0.05$.

the CON group had elevated levels of γ-butyrobetaine, but breast muscle also showed higher content although to a lesser extent.

The CAR group had a markedly higher level of carnitine in the crop milk than the CON group (Tab. VII). The carnitine level in the breast muscle of CAR was also elevated but because of high individual variation, the level of significance was not reached.

In all tissues except for crop milk the average γ-butyrobetaine level was lower due to L-carnitine supplementation, but this was only significant for the breast muscle. Acetylcarnitine concentrations did not differ between treatments.

3.3.5. Dry matter and protein content of the crop milk (mean ± standard deviation)

No between treatment differences were seen in the dry matter content of the crop milk (CON: 91 ± 4 g·kg^{-1} and CAR: 91 ± 4 g·kg^{-1}). The protein content accounted for 533 ± 26 g·kg^{-1} dry matter for CON and 541 ± 17 g·kg^{-1} dry matter for CAR but this also was not significant.

3.3.6. Protein content of heart, liver and breast muscle

No between treatment differences were observed (Tab. VIII). The protein level increased significantly from liver to heart to breast muscle tissue ($p < 0.001$).

4. DISCUSSION

The breeding pattern was in accordance with observations by Shetty et al. [22].

The sex-linked evolution of the weight loss in Exp1 might be related to the sex-differentiated production of crop milk: in a study by De Cock et al. [7] the crop of male pigeons contained crop milk up to 16 days after hatching whereas in female pigeons crop milk was only found up to 3 days after hatching.

The gradual rise with age of feed intake within a nest is explained by an increased intake of the parent pigeons because pigeons are a nidicole species, which implies that the squabs had no access to the feed before they were able to leave the nest. In a study by Vandeputte-Poma and van Grembergen [25] the leaving of the nest occurred between 22 and 25 days of age. The high standard errors in feed intake from d17 on, most likely reflect the cease of crop milk feeding and the concomitant drop in feed intake by the parent pigeons forces the squabs to develop autonomous feed intake. It is not excluded that the trend to a higher feed intake of the L-carnitine group after day 17 was due to an enhanced feed intake of the more developed squabs from the CAR group but this effect was not significant since it could be masked by the lower feed intake of the parents after ending the crop milk production.

The enhancement of growth due to L-carnitine supplementation is seen in other

Table VIII. Protein levels in heart, liver and breast muscle in Exp2 ($n = 12$).

	CON $N = 6$	CAR $N = 5$	*P*-value	SEM
Heart, mg protein·g^{-1} fresh weight	19.9	20.4	NS	0.3
Liver, mg protein·g^{-1} fresh weight	19.6	18.7	NS	0.3
Breast muscle, mg protein·g^{-1} fresh weight	21.9	21.9	NS	0.4

CAR = carnitine supplemented group; CON: control group; NS = not significant at $p < 0.05$.

animal species, for example in pigs [19], although other authors have not found a significant growth improvement in broilers and turkeys [1]. In the present study however, growth improvement was only seen when parent pigeons were supplemented with L-carnitine. In mammals, several publications have shown the importance of exogenous L-carnitine supply to neonate animals [3]. Rinaudo et al. [21] and Chiodi et al. [6] both showed significantly lower levels in post-hatch chick tissues when compared to adult chickens. Using data from Vandeputte-Poma and Desmeth [26], we determined that the daily crop milk intake by 1–5 d old squabs can be up to 72% of their body weight. Even if only the average free carnitine content in the non-supplemented crop milk of Exp2 and Exp3 is taken into account (i.e. 138 nmol·mL^{-1}), the daily L-carnitine intake by the squabs can be calculated to be about 100 μmol per kg body weight. In a study by Cederblad and Svenningsen [5], the highest carnitine intake through breast milk in neonate infants was only 13 μmol per kg body weight. This led to via conclusion that the carnitine supply through crop milk feeding is quite high. This is supported by the present data showing that L-carnitine supplementation to crop milk fed squabs has no effect on growth. Moreover, supplementation of L-carnitine in sows during lactation does not result in higher piglet yield [16].

Hence, the demonstrated effects in squabs of L-carnitine supplementation in parent pigeons find their explanation in an altered composition, quantity or duration of crop milk feeding. Supplementation of L-carnitine in sows during gestation results in higher sow weights and higher piglet and litter weights at parturition and (therefore) at weaning [17]. It is not clear from this sow trial whether other milk production parameters than milk composition, for example duration or quantity, were affected by L-carnitine supplementation. The lack of significant L-carnitine effects on the dry matter and protein content of crop milk indicates that the major components of the crop milk had not markedly changed. Although quantification of the crop milk could not be performed in the present setup, increased crop milk production is the best possible explanation for the elevated squab growth.

It is remarkable that the growth effect of L-carnitine could be seen in a period when the L-carnitine supplementation had already stopped. This might suggest a beneficial effect of L-carnitine on the duration of the crop milk production. Vandeputte-Poma [24] found that the duration of crop milk feeding is positively correlated with the growth of the squabs. Crop milk contains a growth factor that also enhances growth in other animal species [2].

Since the applied HPLC method has been tested for accuracy and precision, the high variability in the (acyl)carnitine and γ-butyrobetaine data should be contributed to the combination of a low number of animals and individual variation. Unfortunately, the intensive character of the trials did not allow a high number of animals to be used and some crop-milk samples had to be pooled to obtain sufficient amounts. Still, both supplementation of parent pigeons and squabs resulted in a clear increase of carnitine content in crop milk taken from the squabs. The uptake of this extra L-carnitine was proven by the increased carnitine level in the breast muscle of the squabs from the supplemented pigeons, even though the supplementation had been stopped for more than two weeks before sampling. Moreover, a considerable fall in the γ-butyrobetaine level in the breast muscle is evidence for a negative feedback mechanism on the biosynthesis of L-carnitine, since γ-butyrobetaine is the last step in this biosynthesis before L-carnitine [4]. The absence of an impact on acetylcarnitine of the L-carnitine supplementation may indicate that the acylation activity of L-carnitine is subject to homeostasis and is therefore not able to induce beneficial economic effects through, for instance, an enhancement of

ATP production in the Krebs cycle. Because the breast muscle was the only kind of tissue where these differences were shown (Tab. VII), the storage capacity of the heart, liver and plasma must have other kinetics that imply either a higher resistance to the uptake or a higher clearance rate of L-carnitine. This is in agreement with Rebouche and Paulson [20] who found a steep carnitine gradient between muscle and extracellular fluid and similar but less steep gradients between other tissues and extracellular fluid [20].

Direct supplementation of L-carnitine to pigeon squabs does not induce metabolic changes leading to growth improvement, possibly because of a negative feedback regulation on the carnitine precursor γ-butyrobetaine. The enhanced squab growth due to L-carnitine supplementation in breeding pigeons may be motivated by increased crop milk production.

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