Dietary L-carnitine supplementation increases antigen-specific immunoglobulin G production in broiler chickens

Jan Mast*, Johan Buyse and Bruno M. Goddeeris

Laboratory for Physiology and Immunology of Domestic Animals, Katholieke Universiteit Leuven, Kardinaal Mercierlaan 92, B-3001 Heverlee, Belgium

(Received 22 January 1999 – Revised 7 June 1999 – Accepted 16 August 1999)

The usefulness of supplementary dietary L-carnitine as an immunomodulator to increase antigen-specific antibody levels was analysed in 2–6-week-old broilers. The chickens received commercial feeds either unsupplemented (starter feed 17·8 mg carnitine/kg, finisher diet 22·9 mg carnitine/kg) or supplemented with L-carnitine (100 mg carnitine/kg added to feed). At 14 d of age, both groups were distributed in equal numbers and sex ratios over two environmentally controlled chambers where temperature (28°) was either reduced immediately to 20°, or gradually to 22° at 36 d of age. Antigen-specific immunoglobulin (Ig)M, IgG, IgA and total Ig responses were measured following two immunizations with bovine serum albumin (BSA). The typical BSA-specific IgM responses followed by IgG responses to the primary immunization were boosted by the secondary immunization. The kinetics of these responses were not altered by L-carnitine treatment. However, BSA-specific total Ig and IgG, but not IgM, responses were significantly increased by dietary L-carnitine supplementation, after both the primary and the secondary immunization. No significant influence of the sex of the chicks or the imposed environmental temperature on Ig responses was found. Temperature treatment and sex, but not L-carnitine supplementation, did significantly influence body-weight gain: cockerels were heavier than females and this became most evident in the second half of the rearing period. Further, lowering the temperature increased body weight. In conclusion, dietary L-carnitine supplementation appeared to be beneficial in enhancing specific humoral responses on vaccination.

L-Carnitine: Broiler chickens: Antibody response

L-Carnitine (β-OH-(γ-N-trimethylamino)-butyrate) is a water-soluble quaternary amine. It is synthesized endogenously from the essential amino acids lysine and methionine (Bieber, 1988). In normal physiological and nutritive conditions, L-carnitine requirements are, however, largely covered by dietary sources.

The major metabolic role of L-carnitine appears to be the transport of long-chain fatty acids into the mitochondria for β-oxidation (Coulter, 1995). Thus, dietary L-carnitine supplementation could improve fatty acid and energy utilization and therefore gain and feed efficiency, especially in young animals where synthesis is insufficient to meet endogenous requirements (Gropp *et al.* 1994). L-Carnitine supplementation increased body-weight gain, reduced carcass fat and improved feed conversion in weaning pigs (Weeden *et al.* 1991) and broiler chickens (Von Lettner *et al.* 1992; Rabie *et al.* 1997*a*,*b*). Schumacher *et al.* (1993) and Gropp *et al.* (1994) demonstrated in piglets and baby quail that weight gain and feed conversion efficiency were more clearly affected when lysine and methionine supply was marginal than when these amino acids were supplied to requirements. Barker & Sell (1994) observed, however, no influence of dietary L-carnitine supplementation on performance and carcass composition of broilers and young turkeys fed on low- or high-fat diets.

In addition to its role in the oxidation of fatty acids, L-carnitine has been found to exhibit immunomodulatory effects. *In vitro*, L-carnitine supplementation increased the proliferative responses of both murine and human lymphocytes following mitogenic stimulation (Cavazza, 1983). Further, the defective proliferative capability of peripheral blood lymphocytes of elderly people and patients with acquired immune deficiency syndrome was considerably improved by L-carnitine treatment (De Simone *et al.* 1982, 1993, 1994; Franceschi *et al.* 1990). Furthermore, there are indications that L-carnitine increases polymorphonuclear chemotaxis (De Simone *et al.* 1982). On activation of human mononuclear phagocytes, more than 50 % of

Abbreviations: BSA, bovine serum albumin; DPI, days post-immunization; Ig, immunoglobulin.

* **Corresponding author:** Dr Jan Mast, fax +32 2 375 0979, email jamas@var.fgov.be

acetylcarnitine is transformed into L-carnitine, indicating that acetylcarnitine plays an important metabolic role when mononuclear phagocytes initiate an immune response (Kurth *et al.* 1994).

Addition of L-carnitine to cultured mouse hybridoma cells stimulates growth and antibody production (Typlt *et al.* 1991; Berchiche *et al.* 1994). Higher levels of antibodies specific to influenza and pneumococcal vaccines were produced in L-carnitine-supplemented mice than in control mice fed on an unsupplemented diet (Shug & Gravenstein, 1996).

In the present study, the use of supplementary dietary L-carnitine as an immunomodulator to increase antigen-specific antibody levels of broiler chickens was explored.

Materials and methods

Chickens, management and experimental design

Sexed 1-d-old broiler chicks (*n* 840) from a commercial line (Ross 208) were obtained from a local hatchery (Avibel, Halle-Zoersel, Belgium). The chicks were housed by sex in twenty-eight litter pens (1 m × 0·8 m; thirty chicks per pen) arranged in an environmentally controlled room. Wood shavings were used as litter and the lighting schedule provided 23 h light/d. Half the chicks of each sex were assigned randomly to seven pens receiving the starter diet (control; Table 1) and the other half (L-carnitine) to pens receiving the chick starter diet supplemented with 100 mg L-carnitine (Lonza, Basel, Switzerland)/kg. The L-carnitine concentration in the unsupplemented starter diet (17·8 mg/kg) was calculated based on L-carnitine contents in feedstuffs given by Baumgartner & Blum (1997). The level of supplemental L-carnitine was based on the level used by Rabie *et al.* (1997*a*) that improved body-weight gain and decreased abdominal fat in broiler chickens. Treatments were randomized in the room. Temperature was initially set at 36° and was gradually reduced to 28° at 14 d of age. Water and feed were provided *ad libitum* during the entire experiment.

At 14 d of age, the four groups (male, 100 mg L-carnitine/kg; male, no supplementation; female, 100 mg L-carnitine/kg; female, no supplementation) were distributed in equal numbers over two identical environmentally controlled rooms. In one room, temperature was reduced gradually until 22° was reached at 36 d of age. In the other room, ambient temperature was kept constant at 20° from day 14 until the end of the experiment. This low-temperature regimen was included to increase metabolic stress, which might increase L-carnitine requirements. Within each temperature programme, the chickens that received the control starter diet were now supplied with a commercial finisher diet (Table 1). The chickens that previously received the L-carnitine-supplemented starter diet now received a finisher diet supplemented with 100 mg L-carnitine/kg. The calculated L-carnitine concentration in the unsupplemented finisher diet was 22·9 mg/kg. Each pen (1 m × 0·8 m) was provided with a 0·8 m feeder and two drinking nipples and contained fifteen chickens initially.

This experimental design resulted in a 2^3 factorial design with environmental temperature (normal or low temperature programme), sex (male or female) and L-carnitine (no supplementation or 100 mg/kg) as main factors and seven replications (pens) per treatment. Sex and dietary treatments were randomized within each room.

Table 1. Composition and proximate analysis of the basal starter and finisher diets (g/kg)

Ingredients	Starter diet	Finisher diet
Wheat	350·2	384·5
Yellow maize	150·0	100·0
French peas	150·0	120·0
Soyabean meal (500 g/kg)	125·0	34·0
Soyabeans	100·0	100·0
Sunflower meal (280 g/kg)		5·0
Oilcake meal	20·0	55·0
Rapeseed meal		26·0
Animal meal	65·0	105·0
Fish meal	10·0	10·0
Destruction fat	12·0	18·0
Fatty acids		10·0
Limestone	2·4	
DL-Methionine	2·3	1·3
Lysine	2·1	0·7
Choline	0·5	0·5
Common salt	1·5	
Vitamin and mineral premix	4·0*	4·0†
Wheat enzyme preparation	4·0	4·0
Sodium bicarbonate		1·0
Sepiolite		20·0
Total	1000·0	1000·0
Calculated composition		
Crude protein	222·2	214·2
Crude fibre	29·7	31·5
Crude fat	58·2	81·3
Starch + sugars	428·4	408·7
Lysine	11·8	10·4
Methionine + cystine	8·8	7·6
Tryptophan	2·2	2·1
Threonine	7·1	6·7
Choline	1·5	1·5
Essential fatty acids	19·9	24·3
K		7·1
Ca	9·0	7·5
P (total)	7·0	6·0
P (available)	4·9	4·3
Cl	2·6	2·3
Na	1·5	1·9
Metabolizable energy (MJ/kg)	12·1	12·7
L-Carnitine (mg/kg)	17·8	22·9
Moisture	117·9	110·0

* Provided (mg/kg diet): α-tocopherol 30, menadione 1·5, thiamin 1·5, riboflavin 4, pantothenic acid 10, niacin 30, biotin 0·07, cyanocobalamin 0·02, pyridoxine 2, pteroylmonoglutamic acid 1, Fe 90, Cu 22, Zn 50, Mn 80, Co 0·2, I 0·8, Se 0·2, retinol 3·78, cholecalciferol 0·062.

† Provided (mg/kg diet): α-tocopherol 30, menadione 2, thiamin 2, riboflavin 4, pantothenic acid 10, niacin 30, biotin 0·05, cyanocobalamin 0·12, pyridoxine 2, pteroylmonoglutamic acid 1, Fe 90, Cu 22, Zn 50, Mn 80, Co 0·2, I 0·54, Se 0·2, retinol 3, cholecalciferol 0·062.

At 15 d of age, one chicken per pen was randomly assigned and immunized subcutaneously in the neck with 1 mg bovine serum albumin (BSA) in an emulsion of PBS (2 mg/ml) and Freund's complete adjuvant (50 : 50, v/v). A subcutaneous booster immunization with 1 mg BSA in an emulsion of PBS (2 mg/ml) and Freund's incomplete adjuvant (50 : 50, v/v) was given 21 d later, at 36 d of age. Serum samples were collected from individually identified and weighed animals before the primary immunization, at 8, 10, 13 and 21 d post-immunization (DPI), and 2 and 4 d after

the booster immunization. The other chickens were used to study the effect of L-carnitine on endocrine and performance variables (J Buyse, G Jansens and E Decuypere, unpublished results).

Quantification of bovine serum albumin-specific humoral responses

BSA-specific immunoglobulin (Ig)M, IgG and IgA responses were measured by isotype-specific ELISA as described by Mast *et al.* (1997). In addition, BSA-specific total Ig was measured similarly using, however, a peroxidase-labelled sheep polyclonal antibody against chicken Ig (Sigma Chemical Co., St Louis, MO, USA).

Statistical analysis

The effects of sex and L-carnitine supplementation on the responses before immunization were analysed by ANOVA using the general linear models procedure of the SAS® system (Version 6, 1986; Statistical Analysis Systems Institute Inc., Cary, NC, USA). Significance of differences between the means of these responses was determined by Tukey's Studentized range test.

This experiment is a typical repeated measures experiment, consisting of chickens randomly assigned to the treatment groups, and with the weight and BSA-specific antibody responses measured on each animal over a sequence of time points (DPI). Therefore, the mixed model methodology, provided by the MIXED procedure (PROC MIXED) of the SAS® system (release 6·11, 1996; Statistical Analysis Systems Institute Inc.) was used to analyse these repeated measures data as described by Littell *et al.* (1998). Using PROC MIXED, the covariance structure of the weights and responses following immunization was modelled and the best fitting structure was selected based on the Schwartz Bayesian goodness-of-fit criterion. Given this covariance structure, fixed effects were tested and means with their standard errors were calculated and compared using the LSMEANS and ESTIMATE statements of PROC MIXED respectively.

Results

To assess the influence of dietary L-carnitine supplementation on the humoral immune responses of broiler chickens, the total Ig, IgM, IgG and IgA responses against the thymus-dependent antigen BSA were evaluated.

As a control, BSA-specific IgM, IgG and total Ig levels were measured before immunization. The recorded absorbances for IgG (mean 0·019, SE 0·003), total Ig (mean 0·015, SE 0·003) and IgM (mean 0·053, SE 0·005) were relatively low and were not significantly influenced by the sex or the L-carnitine treatment of the chicks.

Variance at individual times and correlation between responses at different times post-immunization on the same animal were modelled in PROC MIXED by the indicated covariance structures. Of all structures examined, the unstructured covariance, which makes no assumptions regarding equal variances or correlations, gave the highest Schwartz Bayesian criterion values for total Ig, IgG and IgM (Table 2). Therefore, unstructured covariance was used to estimate treatment effects.

Table 2. Comparison of covariance structures using the Schwarz Bayesian goodness-of-fit criterion* (SBC)

	SBC for dependent variable			
Covariance structure	IgM	IgG	Ig	Weight
Simple	68	–130	–227	–2347
Compound symmetry	66	–74	–174	–2298
Unstructured	153	–50	–157	–2072
Autoregressive(1)	66	–79	–189	–2249
Autoregressive(1) + residual error	64	–73	–176	–2249
Toeplitz	74	–71	–161	–2344
Banded Toeplitz	65	–95	–201	–2288

Ig, immunoglobulin.
* Schwarz Bayesian goodness-of-fit criterion: the larger the value, the better the covariance structure.

The sex of the chicks and the imposed environmental temperature did not significantly influence IgM, IgG and total Ig responses. Likewise, interactions between sex, temperature, L-carnitine supplementation and DPI did not significantly influence IgM, IgG and total Ig responses. Therefore, the data were pooled for further analysis and presentation of the L-carnitine effect.

Across treatments, the effect of DPI on BSA-specific total Ig, IgG and IgM responses was highly significant ($P < 0·0001$). As expected, the highest BSA-specific IgM responses were recorded 8 d after the primary immunization (Fig. 1). Afterwards, these IgM responses rapidly decreased and then increased again 4 d after the booster immunization. BSA-specific total Ig (Fig. 2) and IgG (Fig. 3) responses

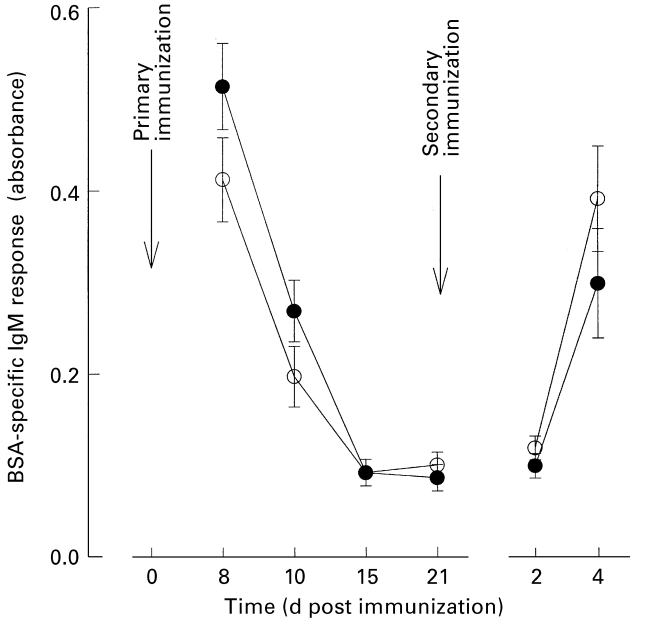

Fig. 1. Bovine serum albumin (BSA)-specific immunoglobulin M (IgM) responses of broiler chickens receiving dietary L-carnitine (●) and control chickens (○), following two immunizations with BSA. Values are means for twenty-eight chickens, with their standard errors represented by vertical bars.

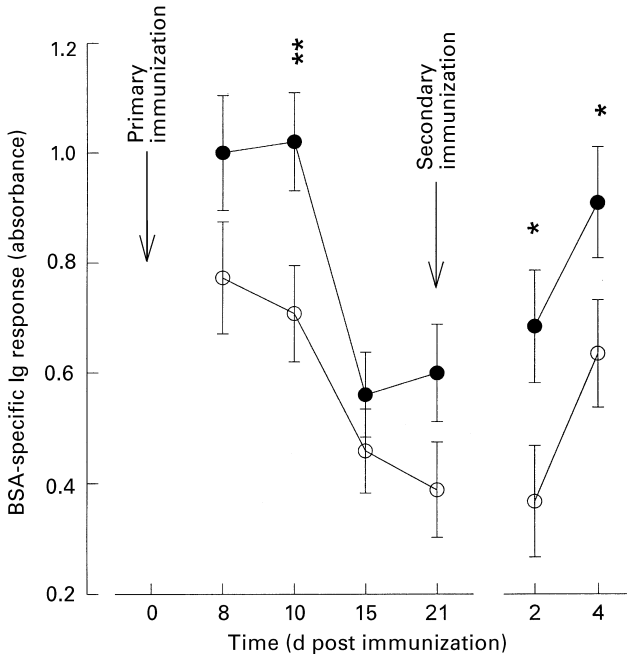

Fig. 2. Bovine serum albumin (BSA)-specific total immunoglobulin (Ig) responses of broiler chickens receiving dietary L-carnitine (●) and control chickens (○), following two immunizations with BSA. Values are means for twenty-eight chickens, with their standard errors represented by vertical bars. Mean values for L-carnitine-fed chickens were significantly different from those for the controls: $*P < 0.05$, $**P < 0.01$.

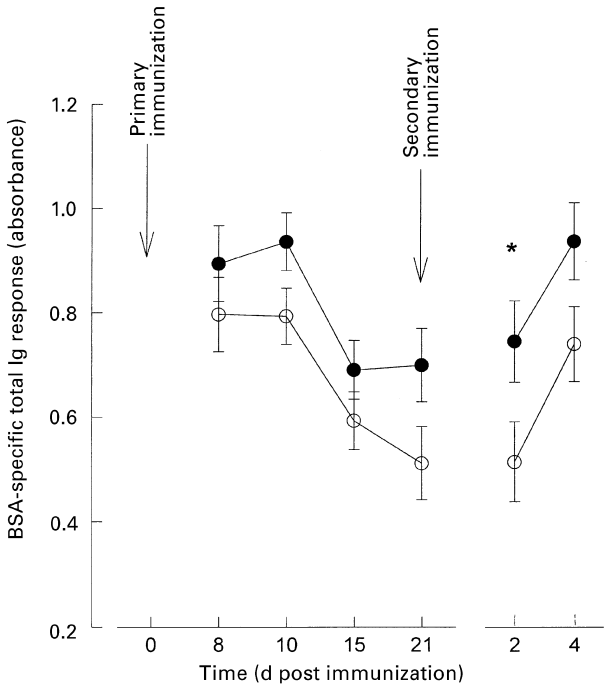

Fig. 3. Bovine serum albumin (BSA)-specific immunoglobulin G (IgG) responses of broiler chickens receiving dietary L-carnitine (●) and control chickens (○), following two immunizations with BSA. Values are means for twenty-eight chickens, with their standard errors represented by vertical bars. Mean value for L-carnitine-fed chickens was significantly different from that for controls: $*P < 0.05$.

showed similar kinetics but remained high until 10 d after the primary immunization.

Across DPI, dietary L-carnitine supplementation significantly increased BSA-specific total Ig ($P = 0.02$; Fig. 2) and IgG ($P = 0.03$; Fig. 3), but not IgM ($P = 0.70$; Fig. 1) levels. Mean BSA-specific IgM responses were numerically, but not significantly, higher at 8 and 10 DPI in L-carnitine-treated birds, but were equal or lower afterwards.

The interactions between DPI and L-carnitine treatment were not significant and comparison of means between DPI further confirmed that the kinetics of IgM, IgG and total Ig responses were not influenced by L-carnitine supplementation. No serum BSA-specific IgA could be detected.

When the covariance structure of the individual weights of the chickens was modelled using PROC MIXED, the unstructured covariance gave the highest Schwartz Bayesian criterion value (Table 2) and was thus used to estimate fixed effects. In contrast to antigen-specific Ig, the body weight was not influenced by L-carnitine supplementation. Only sex, the interaction sex × age and temperature treatment significantly influenced the body weight of the chickens (Fig. 4). The body weight of male broilers was higher than that of female broilers (sex effect $P \leq 0.002$) and this difference became apparent from the second half of the rearing period resulting in a significant interaction between sex and age ($P \leq 0.001$). Lowering the environmental temperature increased body weight ($P \leq 0.03$). These findings were corroborated by the results of the parallel experiment focusing on zootechnical and endocrine variables where fourteen times more chickens were examined (J Buyse, G Jansens and E Decuypere, unpublished results). In this experiment, feed uptake and conversion, as body-weight gain, remained unaltered by L-carnitine supplementation. Further, no increased mortality or clinical signs of disease were observed.

Discussion

Dietary L-carnitine supplementation was shown to exert an immunomodulatory effect on antigen-specific total Ig and IgG responses. This finding is consistent with the observations of Shug & Gravenstein (1996) who claimed that the use of L-carnitine or its precursors stimulates an improved antigenic response in mice, and corroborates the results of Typlt *et al.* (1991) and Berchiche *et al.* (1994) who found that L-carnitine stimulates antibody production by murine hybridoma cells.

Because of the absence of BSA-specific IgA responses, the low IgM levels in serum in comparison with IgG (Benedict & Berestecky, 1987) and the identical kinetics of BSA-specific total Ig and IgG (Fig. 2 and Fig. 3), it can be assumed that the observed effect of L-carnitine supplementation on BSA-specific total Ig largely reflects the influence of L-carnitine supplementation on IgG. The increased BSA-specific IgG responses were not transient but continued from 8 d after the primary immunization until at least 4 d after the booster immunization (final day of analysis). This, and the observation that L-carnitine supplementation had little or no effect on BSA-specific IgM responses, might indicate that L-carnitine supplementation

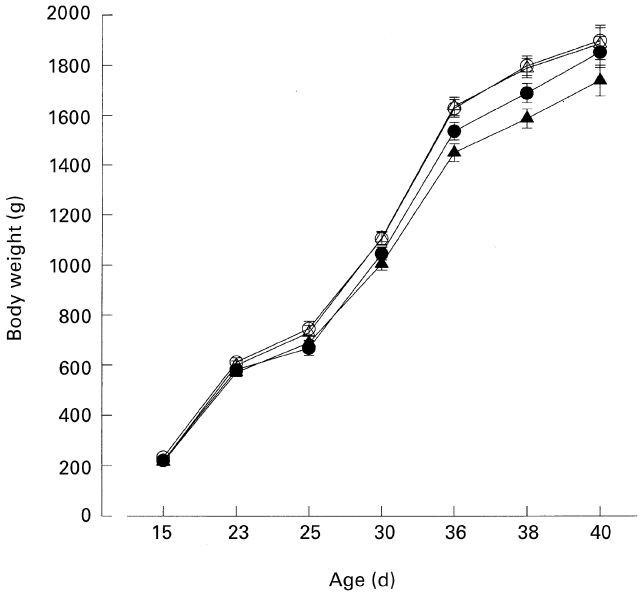

Fig. 4. Change in body weight with age in male (○, △) and female (●, ▲) broiler chickens kept at a low environmental temperature (○, ●) or at a normal environmental temperature (△, ▲). Values are means for fourteen chickens, with their standard errors represented by vertical bars.

does not, as most adjuvants, enhance the onset of immune responses but specifically interferes in IgG production by plasma cells or in the isotype-switching towards IgG.

The compensation of an L-carnitine deficiency at the level of the involved immunocompetent cells by dietary L-carnitine supplementation may explain the observed increase of the specific IgG responses of the broiler chickens. Indeed, during the period of vaccination and the subsequent humoral response, i.e. from 2 to 6 weeks of age, the growth rate of the investigated broilers was maximal (results not shown). This high metabolic rate suggests a concomitant high need for L-carnitine. The high metabolic requirements combined with an endogenous L-carnitine synthesis that has not yet reached its full capacity in young animals (Gropp *et al.* 1994; Janssens & De Wilde, 1994) may lead to L-carnitine deficiency.

Schumacher *et al.* (1993) and Gropp *et al.* (1994) demonstrated in piglets and baby quail that L-carnitine effects are more evident when lysine and methionine + cysteine supply is marginal than when these amino acids are supplied to requirements. In the present experiment, calculated levels of these L-carnitine precursors and of methyldonors such as choline and folic acid were, however, adequate in the experimental diets according to the nutrient requirements for poultry outlined by the National Research Council (1994). Moreover, the unchanged body-weight gain and food conversion efficiency argue against a marginal deficiency of one of these 'L-carnitine sparing' nutrients. Therefore, our findings, and the findings of De Simone *et al.* (1994) that L-carnitine depletion could be found in peripheral blood mononuclear cells from patients with acquired immune deficiency syndrome, although their serum L-carnitine levels were normal, rather indicate that L-carnitine deficiency may be more explicit at the lymphocyte level than in other tissues.

The mechanism(s) accounting for the positive effect of L-carnitine on antibody production is currently not clear. Restoration of the cellular L-carnitine content might have enhanced the lipid metabolism and improved the cellular energy balance. De Simone *et al.* (1994) indicated that L-carnitine decreases the concentrations of cytokines, most notably tumour necrosis factor-α in man. In the rat models of cachexia and septic shock, L-carnitine treatment lowered levels of interleukin-1β, interleukin-6 and tumour necrosis factor-α (Winter *et al.* 1995). These cytokines play a pivotal role in general energy homeostasis, but also in the modulation of antibody responses. Finally, Di Marzio *et al.* (1997) have recently shown that L-carnitine down-regulates acidic sphingomyelinase activity. This enzyme converts sphingomyelin into ceramide, an intracellular messenger molecule inducing apoptosis. In the acquired immune deficiency syndrome model, nutritional L-carnitine supplementation has been shown to reduce ceramide production and apoptosis (Famularo & De Simone, 1995; Cifone *et al.* 1997; Moretti *et al.* 1998). Plausibly, L-carnitine also prevented apoptotic cell death of B and T lymphocytes during the immune responses of broiler chickens, which resulted in higher antibody titres.

Historically, nutrient requirements of domestic animals have been established using growth, feed efficiency and reproduction as criteria (Cook, 1996). Our findings suggest that immune response should be considered when establishing nutritional guidelines, especially with respect to L-carnitine. Indeed, our findings indicate that dietary L-carnitine supplementation may increase antigen-specific IgG responses, while growth performance was not improved in this and similar experiments (Barker & Sell, 1994). A long-lasting increased IgG response as a result of dietary L-carnitine supplementation may be of major practical importance in the enhancement of protective immunity on vaccination. Moreover, because of the positive linear correlation between the amount of specific IgG in the serum and in the eggs of laying hens (Bollen & Hau, 1997), an L-carnitine-mediated increase of serum IgG results in an increased transfer of IgG to the eggs. Thus, L-carnitine supplementation may improve maternal immunity or the yield of commercially extractable antibodies in the egg.

Acknowledgements

The authors wish to thank Klaas Vos and Ingrid Geerts for their technical assistance and Lonza (Basel, Switzerland) for kindly providing L-carnitine.

References

Barker DL & Sell JL (1994) Dietary carnitine did not influence performance and carcass composition of broiler chickens and young turkeys fed low- or high-fat diets. *Poultry Science* **73**, 281–287.

Baumgartner M & Blum R (1997) Typical L-carnitine contents in feedstuffs. In L-*Carnitine in Animal Nutrition*. Basle: Lonza Ltd.

Benedict AA & Berestecky JM (1987) Special features of avian immunoglobulins. In *Avian Immunology: Basis and Practice*, vol. I, pp. 113–125 [A Toivanen and P Toivanen, editors]. Boca Raton, FL: CRC Press.

Berchiche L, Legrand C, Capiaumont J, Belleville F & Nabet P

(1994) Effect of L-carnitine and acylcarnitine derivatives on the proliferation and monoclonal antibody production of mouse hybridoma cells in culture. *Journal of Biotechnology* **34**, 175–183.
Bieber LL (1988) Carnitine. *Annual Reviews of Biochemistry* **57**, 261–283.
Bollen LS & Hau J (1997) Immunoglobulin G in the developing oocytes of the domestic hen and immunospecific antibody response in serum and corresponding egg yolk. *In Vivo* **11**, 395–398.
Cavazza C (1983) Therapeutical method of treating patients with impaired immune system. United States Patent 4 415 588.
Cifone MG, Alesse E, Di Marzio L, Ruggeri B, Zazzeroni F, Moretti S, Famularo G, Steinberg SM, Vullo E & De Simone C (1997) Effect of L-carnitine treatment in vivo on apoptosis and ceramide generation in peripheral blood lymphocytes from AIDS patients. *Proceedings of the Association of American Physicians* **109**, 146–153.
Cook ME (1996) Diet-induced immunosuppression. In *Poultry Immunology*, pp. 317–325 [TF Davison, TR Morris and LN Payne, editors]. Abingdon: Carfax.
Coulter DL (1995) Carnitine deficiency in epilepsy: risk factors and treatment. *Journal of Child Neurology* **10**, Suppl. 2, 2S32–2S39.
De Simone C, Famularo G, Tzantzoglou S, Trinchieri V, Moretti S & Sorice F (1994) Carnitine depletion in peripheral blood mononuclear cells from patients with AIDS: effect of oral L-carnitine. *AIDS* **8**, 655–660.
De Simone C, Ferrari M, Lozzi A, Meli D, Ricca D & Sorice F (1982) Vitamins and immunity: II. Influence of L-carnitine on the immune system. *Acta Vitaminology and Enzymology* **4**, 135–140.
De Simone C, Tzantzoglou S, Famularo G, Moretti S, Paoletti F, Vullo V & Delia S (1993) High dose L-carnitine improves immunologic and metabolic parameters in AIDS patients. *Immunopharmacology and Immunotoxicology* **15**, 1–12.
Di Marzio L, Alesse E, Roncaioli P, Muzi P, Moretti S, Marcellini S, Amicosante G, De Simone C & Cifone MG (1997) Influence of L-carnitine on CD95 cross-linking-induced apoptosis and ceramide generation in human cell lines: correlation with its effects on purified acidic and neutral sphingomyelinases in vitro. *Proceedings of the Association of American Physicians* **109**, 154–163.
Famularo G & De Simone C (1995) A new era for carnitine? *Immunology Today* **16**, 211–213.
Franceschi C, Cossarizza A, Troiano L, Salati R & Monti D (1990) Immunological parameters in aging: studies on natural immunomodulatory and immunoprotective substances. *International Journal of Clinical Pharmacology Research* **10**, 53–57.
Gropp JM, Schumacher A & Schweigert FJ (1994) Recent research in vitamin nutrition with special emphasis to vitamin A, β-carotene and L-carnitine. In *Proceedings of the Meeting of the Arkansas Nutrition Conference*, pp. 124–134. Fayetteville, AR: Arkansas Poultry Federation.
Janssens GPJ & De Wilde RO (1994) The use of exogenous carnitine in farm animals. *Vlaans Diergeneeskundig Tijdschrift* **63**, 172–178.
Kurth L, Fraker P & Bieber L (1994) Utilization of intracellular acylcarnitine pools by mononuclear phagocytes. *Biochimica et Biophysica Acta* **1201**, 321–327.
Littell RC, Henry PR & Ammerman CB (1998) Statistical analysis of repeated measures data using SAS procedures. *Journal of Animal Science* **76**, 1216–1231.
Mast J, Desmidt M, Room G, Martin C, Ducatelle R, Haesebrouck S, Davison TF, Kaspers B & Goddeeris BM (1997) Different methods of bursectomy induce different effects on leukocyte distribution and reactivity. *Archive für Geflügelkunde* **61**, 238–246.
Moretti S, Alesse E, Di Marzio L, Zazzeroni F, Ruggeri B, Marcellini S, Famularo G, Steinberg SM, Boschini A, Cifone MG & De Simone C (1998) Effect of L-carnitine on human immunodeficiency virus-1 infection-associated apoptosis: a pilot study. *Blood* **91**, 3817–3824.
National Research Council (1994) *Nutrient Requirements of Poultry*, 9th revised ed. Washington, DC: National Academy Press.
Rabie MH, Szilagyi M & Gippert T (1997*a*) Effects of dietary L-carnitine supplementation and protein level on performance and degree of meatness and fatness of broilers. *Acta Biologica Hungarica* **48**, 221–239.
Rabie MH, Szilagyi M, Gippert T, Votisky E & Gerendai D (1997*b*) Influence of dietary L-carnitine on performance and carcass quality of broiler chickens. *Acta Biologica Hungarica* **48**, 241–252.
Shug AL & Gravenstein S (1996) Method of stimulating antibody formation. United States Patent 5 569 457.
Schumacher A, Eissner C, Gropp JM, Flachowsky G & Schubert T (1993) Carnitine in fish, piglets and quail. In *Vitamine und weitere Zusatzstoffe bei Mensch und Tier: IVth Symposium*, pp. 407–412 [G Flachowsky, editor]. Jena: Friedrich-Schiller Universitat.
Typlt H, Claus R & Nitzsche K (1991) Influence of carnitine on the growth and productivity of murine hybridoma cells. *Journal of Biotechnology* **18**, 173–175.
Von Lettner F, Zollitsch W & Halbmayer E (1992) Use of L-carnitine in the broiler ration. *Bodenkultur* **43**, 161–171.
Weeden TL, Nelsen JL, Hines RH, Li DF & Swanson JA (1991) The effect of L-carnitine on the utilisation of soybean oil fed to early weaned pigs. *Journal of Animal Science* **68**, 374 Abstr.
Winter BK, Fiskum G & Gallo LL (1995) Effects of L-carnitine on serum triglyceride and cytokine levels in rat models of cachexia and septic shock. *British Journal of Cancer* **72**, 1173–1179.