Differential responsiveness between broiler and layer embryos upon different doses of L-carnitine administration. 1. Hatchability and blood parameters

Unterschiedliche Reaktion von Masthühner- und Legehennenembryonen auf verschiedene Dosierungen von L-Carnitin. 1. Brut und Blutparameter

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Introduction

Several factors in newly hatched chicks can influence post-hatch growth including embryonic parameters. For instance, yolk sac is an important source of energy in developing embryo. Energy of chick during the first days of life is provided by oxidation of fatty acids of yolk sac content (Puvadolpirod et al., 1997). It is also assumed that yolk sac is involved in initiation of the growth process for chicks (Bigot et al., 2001) and carries immunoglobulins which are transferred from hen to chick. Efficient utilization of yolk sac content can be enhanced by administration of substances that are involved in fatty acid metabolism. L-carnitine is well known for its enhancing metabolic use of fatty acids (Rebouche, 1992 and Heo et al., 2001) and in addition for its anti-oxydant activity. Indeed, L-carnitine enhances long chain fatty acids’ uptake through mitochondrial membrane. According to Casillas and Newburgh (1969), L-carnitine facilitates the transfer of fatty acyl groups from yolk into tissues of embryonic chicks via the yolk sac membrane. At d 18 of incubation, the ratio of esterified short chain L-carnitine/free L-carnitine of avian embryos is highest in the tissues suggesting that fatty acid oxidation for energy production in embryos is important (Rinaudo et al., 1991). But, freshly laid eggs have low concentrations of L-carnitine (Chiodi et al., 1994) and chicken embryo has a limited capacity to synthesize L-carnitine during incubation (Casillas and Newburgh, 1969). However, during hatching process, energy needs of the embryo increase speedily. Therefore, L-carnitine level could be a limiting factor for oxidation of fatty acids during hatching process. Thus, in ovo administration of L-carnitine during the last stage of incubation of chicken eggs may 1) provide additional energy source for hatching activities and 2) lead to changes in some physiological parameters during peri-natal period.

The aim of this study was to investigate the effects of in ovo injection of different doses of L-carnitine in egg air chamber at d 18 of incubation on hatchability and physiological parameters. Also, comparison effects of administration of L-carnitine between layer-type and broiler embryos/chicks were investigated.

Material and Methods

Experimental design

Hatching eggs from Ross broiler breeders and Isa Brown layer breeders of 35 wk old (630 eggs per line) provided by Belgabroed n.v. (Hoogstraten, Belgium) were used. The eggs were incubated at standard incubation conditions of dry bulb temperature of 37.6°C and wet bulb temperature of 29°C. During the first 18 d of incubation, the eggs were turned once every hour at an angle of 90°. Prior to setting for incubation and at d 18 of incubation, hatching eggs were weighed for egg weight loss determination. At embryonic d 6 and every two days from d 10 to d 18 of incubation, sample of eggs were opened to determine embryo weight according to genotype. At d 18 of incubation, incubated eggs from each strain were divided into 4 groups of 150 eggs each. These groups were:

- Control: eggs without any treatment;
- Saline: eggs injected with saline solution of 0.9%;
- LC500: eggs injected with L-carnitine of 500 μmol;
- LC1000: eggs injected with L-carnitine of 1000 μmol.

At the end of incubation (21 d) all the hatched chicks were recorded and were classified as chicks of optimal or sub-optimal quality. The chicks were reared for 7 d.

Protocol of L-carnitine administration

Saline or L-carnitine solutions were injected in egg air chamber after candling. Only eggs with evidence of living embryos received 100 μL of solution. A needle of 18G was used to drill two holes through the shell above the air chamber in order to decrease the pressure within and thereby facilitating the retention of the injected solution. After injection into one of the holes, both holes were sealed with adhesive tape and the egg was placed in the hatching baskets.

Blood sampling

At hatch, blood samples were obtained from 30 chicks per treatment by decapitation. The same numbers of chicks, from injected or non-injected eggs, were again used for blood sampling at d 7 post-hatch. Blood samples were used for measurements of triglyceride, total protein, uric acid, triiodothyronine (T3), thyroxine (T4) and corticosterone concentrations.

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**T3, T4 and corticosterone analyses**

T3 and T4 concentrations were measured in plasma samples by radioimmunoassay (RIA) as described previously (Huybrechts et al., 1989 and Darras et al., 1992). Intra-assay coefficients of variation were 4.5 and 5.4% for T3 and T4, respectively. Antiserum, T3 and T4 standards were purchased from Byk-Belga (Germany). Corticosterone was measured using a commercially available double antibody RIA-kit from MP Biomedicals, Illkirch, Cedex, France. The intra-assay coefficient of variance was 3.9%. All samples were run in the same assay in order to avoid inter-assay variability (Decuyper et al., 1983 and Meelis et al., 1989).

**Triglyceride, glucose, total protein, uric acid analyses**

Blood samples collected from day-old and 7-day-old chicks were used for glucose, triglycerides, total protein and uric acid measurements. Plasma triglyceride (VetTest 9820377), plasma total protein (VetTest 9820376), plasma glucose (VetTest 9820359) and plasma uric acid (VetTest 9820378) concentrations were determined using the VetTest 8008 analyzer (Idexx Laboratories Inc, U.S.A.). The apparatus is based on dry chemical technology and colorimetric reaction. Sample analysis is carried out on selective testing discs (Idexx Laboratories Inc, U.S.A.) by means of a laser reading the bar codes.

**Hatchability and chick quality**

At the end of incubation (21.5 d), all the eggs that failed to hatch were opened for macroscopically analysis in order to classify them as “unfertile eggs” or eggs containing dead embryos. Then, hatchability of fertile eggs was calculated as shown in formula (1) according to genotype × L-carnitine treatments. For chick quality measurement, all the hatched chicks were examined macroscopically in order to identify the different characteristics that can be associated with good, average, or poor quality chicks based on scoring method described by Tona et al. (2003). Then, proportion of chicks of high quality was calculated as shown in formula (2).

\[
(1) \text{Hatchability} = 100 \times \frac{\text{Number of hatched chicks}}{\text{Number of fertile eggs}}
\]

\[
(2) \text{Chick quality} = 100 \times \frac{\text{Number of chicks of high quality}}{\text{Number of hatched chicks}}
\]

**Statistical analysis**

The data were processed with the statistical software package SAS Version 8.23. Generalized linear regression was used to analyze egg and embryo/chick weights, egg weight loss, plasma triglyceride, total protein, uric acid, T3, T4, corticosterone concentrations in relation to genotype × L-carnitine treatments. When the means of the general model were statistically different, then the means were further compared using Tukey’s test. In a second analysis, logistic regression model was used to analyze hatchabilities and percentages of chick of optimal quality according to the treatments. For all analyses, P value of 0.05 was retained as the degree of significance. The model was:

\[
Y_{ijk} = \mu + \alpha_i + \tau_j + (\alpha\tau)ij + e_{ijk};
\]

where $Y_{ijk}$ = Triglyceride, glucose, total protein, uric acid, T3, T4 or corticosterone concentrations of chick k from genotype i and L-carnitine administration j according to developmental stage, $\mu$ is the overall mean, $\alpha_i$ the main effect of genotype i, $\tau_j$ the main effect of L-carnitine administration j, $(\alpha\tau)$ the interaction between genotype and L-carnitine administration and $e_{ijk}$ is the random error term.

**Results**

**Egg and embryo weights**

Egg weights and egg weight loss up to d 18 of incubation were not affected by genotype (data not shown). Figure 1 shows embryo weights between d 6 and d 18 of incubation according to genotype. At embryonic day (ED) 6 and ED 10, layer-type and broiler embryo weights were comparable. But, from d 12 onwards, broiler chicks grew faster than layer-type chicks (P < 0.001).

**Effects of L-carnitine administration and genotype on hatchability and chick quality**

Table 1 shows the effects of L-carnitine administration on embryonic mortality, hatchability and chick quality in relation to genotype. Irrespective of treatments, hatchability and percentage of chicks of optimal quality were higher while embryonic mortality was lower in Ross eggs compared to Isa Brown eggs (P < 0.05). In both genotype groups, eggs of LC1000 groups had the lowest hatchability and percentage of chicks of optimal quality compared to control, saline and LC500 groups (P < 0.05). In both lines, hatchabilities and percentages of chicks of optimal quality of eggs injected with saline solution were comparable with control and LC 500 groups.

**Effects of L-carnitine administration and genotype on triglyceride levels**

Triglyceride concentrations according to treatment, developmental stages and genotype are shown in Table 2. At
hatch, triglyceride levels were similar between genotype. In broiler line, triglyceride levels in control eggs were comparable with those of saline group but lower than those of eggs injected with L-carnitine (P < 0.05) while levels were not statistically affected by treatment in layer line. At 7 d post-hatch, triglyceride levels were higher in broiler chicks than layer chicks (P < 0.05). However in layer line, triglyceride concentrations in control eggs were comparable with those of saline group but lower than those of eggs injected with L-carnitine (P < 0.05) while levels were now not statistically different anymore between treatments in broiler line.

**Effects of L-carnitine administration and genotype on total protein levels**

Total protein levels according to L-carnitine administration in relation to genotype are indicated in Table 3. At hatch, total protein levels were comparable between layer-type and broiler chicks. But, in layer-type chicks, total protein concentrations in chicks of LC500 and saline groups were similar but higher (P < 0.05) than those of chick of LC1000 and control groups which were also similar. As for 7 d-old chicks, total protein levels in layer-type chicks were higher than those of broiler chicks (P < 0.001). Layer-type chicks of LC1000 group had lower levels of total protein than those of chicks of LC500, saline and control groups for which total protein levels were comparable. In broiler chicks, total protein levels of chicks injected with L-carnitine were similar but lower (P < 0.05) than those of saline and control groups.

**Effects of L-carnitine administration and genotype on uric acid levels**

Plasma uric acid levels according to genotype and development stages are shown in Table 4. From hatch to 7 d post-hatch, uric acid levels were overall higher in layer-type chicks than those of broiler chicks (P < 0.05). At hatch and within each genotype, uric acid concentrations were not different between treatments. At 7 d post-hatch, uric acid levels were higher in broiler chicks from L-carnitine groups (P < 0.05) than those of control and saline groups which were similar. Moreover, uric acid concentrations increased with L-carnitine dose (P < 0.05). In layer-type chicks plasma uric acid levels of LC500 and saline group were similar but higher (P < 0.05) than those of LC1000 and control groups which were also similar.

**Effects of L-carnitine administration and genotype on T₃, T₄ and corticosterone levels**

Table 5 shows T₃ levels according to L-carnitine treatment in relation to genotype. Up to 7 d post hatch, T₃ levels were comparable between layer-type and broiler chicks. In layer-type chicks, T₃ levels of chicks of LC500 and saline groups were similar but higher (P < 0.05) than those of chicks of LC1000 and control groups which were similar. As for broiler chicks, T₃ level of control group was lower than those of the three other groups (P < 0.05). At 7 d-old, T₃ levels were not different between groups.

Table 6 indicates T₄ concentrations according to treatment and genotype. For control and saline groups, T₄ levels

### Table 1. Effects of L-carnitine administration at d 18 of incubation on embryo mortality, hatchability and chick quality according to chicken genotype

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Hatchability (%)</th>
<th>Chick of optimal quality (%)</th>
<th>Embryo mortality (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Ross</td>
<td>Isa Brown</td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>97.5ᵃ</td>
<td>92.7ᵃ</td>
<td>96.7ᵃ</td>
</tr>
<tr>
<td>Saline</td>
<td>96.3ᵃ</td>
<td>93.9ᵃ</td>
<td>95.8ᵇ</td>
</tr>
<tr>
<td>LC500</td>
<td>97.8ᵃ</td>
<td>92.5ᵇ</td>
<td>97.1ᵃ</td>
</tr>
<tr>
<td>LC1000</td>
<td>95.2ᵇ</td>
<td>84.2ᵇ</td>
<td>94.5ᵇ</td>
</tr>
</tbody>
</table>

ᵃᵇ Within column, data sharing no common letter are different and * indicates difference between Isa Brown and Ross strains (P < 0.05).

### Table 2. Effects of L-carnitine administration at d 18 of incubation on triglyceride concentrations (mmol/L) according to chicken genotype

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Developmental stage</th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>At hatch</td>
<td>7 d-old</td>
<td>At hatch</td>
<td>7 d-old</td>
</tr>
<tr>
<td>LC 1000</td>
<td>0.720ᵃ ± 0.050</td>
<td>0.700 ± 0.090</td>
<td>0.930 * ± 0.060</td>
<td>0.700ᵃ ± 0.060</td>
</tr>
<tr>
<td>LC 500</td>
<td>0.750ᵃ ± 0.100</td>
<td>0.640 ± 0.050</td>
<td>0.780 ± 0.080</td>
<td>0.620ᵃ ± 0.080</td>
</tr>
<tr>
<td>Control</td>
<td>0.510ᵇ ± 0.020</td>
<td>0.590 ± 0.100</td>
<td>0.750 * ± 0.090</td>
<td>0.420ᵇ ± 0.060</td>
</tr>
<tr>
<td>Saline</td>
<td>0.640ᵇ ± 0.100</td>
<td>0.650 ± 0.050</td>
<td>0.800 * ± 0.080</td>
<td>0.490ᵇ ± 0.100</td>
</tr>
</tbody>
</table>

ᵃᵇ Within column, data sharing no common letter are different and * indicates difference between Isa Brown and Ross strains (P < 0.05).
were higher in layer type chicks than those of broiler chicks (P < 0.05). Up to 7 d post-hatch, T4 levels were not affected by L-carnitine treatment for broiler chicks. But, in layer-type chicks, T4 concentrations of control and saline groups were higher than those of chicks of L-carnitine injected groups (P < 0.05).

Table 7 shows corticosterone levels in function of L-carnitine treatment and genotype. In general, corticosterone concentrations in layer-type chicks were higher or lower (P < 0.05) than those in broiler chicks at hatch or at 7 d post-hatch, respectively. At hatch as well as at 7 d post hatch, chicks of LC500 group had the lowest (P < 0.01) corticosterone concentration while chicks of LC1000 group had the highest (P < 0.001) corticosterone concentration compared with chicks of control and saline groups which had comparable corticosterone levels.

**Discussion**

The results from this study demonstrate that L-carnitine administration at embryonic d 18 changed some of the physiological parameters that were measured in the chicks during the juvenile growth. They also bring out differential effects of *in ovo* L-carnitine administration on hatchability, chick quality and blood parameters of layer-type and broiler chicks. Our results also suggest that *in ovo* administration of L-carnitine has a long lasting effect after hatch.
It is well known that egg weight and embryo weight during incubation are highly correlated. Even though egg weights were similar between genotype, broiler embryo weights were higher than layer embryo weights from d 10 onwards (Figure 1) suggesting that broiler embryo grew more rapidly than layer embryos in the second half of incubation. Because, broiler embryos grew faster than layer embryos, higher hatchability in broiler line compared to layer line may be partly due to a shorter incubation time of broiler embryos than layer embryos. This difference in hatchability suggests that broiler and layer eggs may not have a similar growth trajectory, incubation patterns should be designed according to genotype. Upon in ovo administration of L-carnitine, in this study, administration of 500 μmol of L-carnitine did not affect hatchability or chick quality in either genotype lines. This result is in line with the report of Zhai et al. (2008) who showed that administration of 10 μmol/egg did not affect hatchability in layer hatching eggs. However, administration of 1000 μmol L-carnitine affected negatively hatchability and chick quality only in layer chicks but not in broiler eggs. This negative effect of 1000 μmol L-carnitine in layer embryo may be related to lower body weight of layer embryos and/or to the less advanced developmental stage compared to broiler at the injection stage.

L-carnitine is well known for its action on fat metabolism (Rebouche, 1992 and Heo et al., 2001), enhancing mitochondrial turnover of long chain fatty acids and in addition to its anti-oxidant activity. Surprisingly, triglyceride levels were higher in in ovo L-carnitine treated groups in broiler or layer chicks, respectively at hatch or at 7 d post-hatch indicating that in ovo administration of L-carnitine is involved differentially in chick peri-natal fat metabolism. This difference suggests that fat metabolic rate follows different patterns according to meat type or egg type lines of poultry as already suggested by Sato et al. (2006). In addition, higher triglyceride levels in L-carnitine treated chicks may be due to a negative feedback following fat metabolism induced by L-carnitine injection before hatch. The negative effect of L-carnitine on total proteins levels at 7 d post-hatch may be partly explained by increased mobilization of proteins for growth during this stage. This result is in line with the report of Noibourouko et al. (2010) who pointed out that supplementation of L-carnitine in drinking water leads to reduced total protein levels.

Because corticosterone is involved in maintaining homeostasis, metabolism, and stress (Scott et al., 1981), its levels in chicks can influence chick posthatch growth. Therefore, the significantly higher corticosterone levels in chicks hatched from LC1000 group compared to chicks from LC500, saline and control groups at 7 d post-hatch could influence growth speed of LC1000 chicks. Considering the physiological benefits of administration of 500 μmol/egg of L-carnitine and toxicity effect of the dose of 1000 μmol for layer embryos, L-carnitine can be used as a potential candidate for improving hatchability and post-hatch performance of broilers and layers with a dose not more than 500 μmol.

Since there were differences between saline and control groups with regard to total protein levels in layer chicks at hatch, uric acid concentration in layer chicks at 7 d post-hatch and T3 in both lines at hatch, it is suggested that...
administration of saline solution at 0.9% should be considered as supplementation of additional molecules that can affect embryo physiology. Further investigations are needed for an appropriate medium for in ovo administration of chemical substances. These investigations will focus on post-hatch performances.

In conclusion, L-carnitine administration during embryonic life affected differentially hatchability, chick quality and blood parameters during post-hatch juvenile growth of layer and broiler chicks and this in a dose dependent manner. It is recommended not to inject up to 1000 μmol/egg, especially for layer hatching eggs.

Summary

L-carnitine enhances the transport of long chain fatty acids through mitochondrial membrane. It can be produced by animals' organism from lysine and methionine. However, it was reported that chicken embryos have a limited capacity to synthesize L-carnitine. For this study, hatching eggs from Ross and Isa Brown breeders of 35 wk old (600 eggs per line) were used. At d 18 of incubation, eggs from each genotype were divided into 4 groups i.e. control eggs, Saline (injection of saline solution), eggs injected with L-carnitine of 500 μmol (LC500) or 1000 μmol (LC1000). At hatch and 7 d post hatch, blood samples were collected for triglyceride, glucose, total protein, uric acids, triiodothyronine (T3), thyroxine (T4) and corticosterone concentrations determination.

Results indicate that hatchability and percentage of chick of optimal quality were higher in Ross than Isa Brown. Overall, layer chicks had higher levels of T4, total protein and uric acid than broiler chicks. With regard to L-carnitine injection, eggs of LC1000 groups had the lowest hatchability and this negative effect was more pronounced in Isa Brown eggs. At hatch and 7 d post hatch, control chicks had the lowest levels of triglyceride and T3 but the highest levels of T4. At 7 d-old, the highest and the lowest levels of T4. At 7 d-old, the highest and the lowest levels of corticosterone were obtained in chicks of LC1000 and LC500 groups, respectively, compared to control and saline groups. In conclusion, L-carnitine administration during embryonic life affected differentially hatchability and blood parameters during post-hatch juvenile growth and this in a dose dependent manner.

Key words

Laying hen, genotype, L-carnitine, hatchability, blood parameters

Zusammenfassung

Unterschiedliche Reaktion von Masthühner- und Legehennenembryonen auf verschiedene Dosierungen von L-Carnitin. 1. Brut und Blutparameter


Stichworte

Legehene, Genotyp, L-Carnitin, Brut, Blutparameter

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