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Effects of *in ovo* Administration of L-carnitine on Hatching Events and Juvenile Performance of Layer-type Chick

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Abstract: The effects of *in ovo* injection of L-carnitine on hatchability and juvenile performance of 360 layer-type chicks were investigated. Fertilized eggs were injected in air chamber with L-carnitine (500 and 1000 µmol) dissolved in 0.9% of Saline (NaCl) at d 18 of incubation. Two control groups (non-injected and injected with 0.9% of Saline were also included. Hatched chicks was recorded after every 4 h, beginning at 490 h of incubation and ending at 514 h, for incubation length and hatching spread determination. At the end of incubation, hatched chicks were recorded according to treatment for determination of hatchability. At 3, 7 and 14 d post-hatch, chick body weight (BW) and morbidity were recorded. Also, at d 3 and 7 post-hatch, 14 birds from each of 2 replicate groups within each treatment were used for intestine and yolk sac weight determination. Results indicate that BW, hatchability, or relative intestine weights were not affected by treatment. However, incubation length was longer while hatching spread was shorter in L-carnitine groups compared to control groups. Yolk sac relative weight was decreased by treatment with L-carnitine ($P < 0.05$). Also, the percentage of chicks showing morbidity sign was lower in L-carnitine treated groups from d 7 onwards. The results of the present study suggest that *in ovo* injection of L-carnitine at d 18 of incubation delayed hatching time but resulted in narrower hatching spread, faster utilization of yolk sac content and improved morbidity.

Key words: Layer chick, L-carnitine, hatchability, juvenile performance

INTRODUCTION

A bird's egg is a self-contained life-supporting system for the developing embryo. All nutrients, minerals, energy source and water utilized by the embryo during its incubation are already present in a freshly laid egg, so that the egg requires only warming and periodic turning (Rahn *et al*., 1979). However, nutritional practices of commercial breeder lines often result in unbalanced nutrients for efficient development of embryo. Indeed, hen diets contain low levels of L-carnitine (Buyse *et al*., 2001). Therefore, eggs contain little or no L-carnitine (Chiodi *et al*., 1994). Although L-carnitine biosynthesis increases during embryonic development, its levels are still much lower than those measured in adults because of the low activity of γ-butyrobetaine hydroxylase, the essential enzyme that catalyzes γ-butyrobetaine to L-carnitine (Borum, 1983; Rebouche, 1992). Approximately 90% of the total energy requirement of the developing embryo is derived from fatty acid oxidation of yolk lipids (Noble and Cocchi, 1990), indicating that efficient use of yolk sac content during embryo development may influence incubation time and post-hatch performance. It is well known that L-carnitine is involved in fatty acid metabolism by transportation of long chain fatty acids into the mitochondrial matrix for ß-oxidation (Bremer, 1983). Because of rapid development, a high energy requirement, especially during hatching process, combined with a low ability of L-carnitine synthesis, *in ovo* supplementation of L-carnitine towards the beginning of hatching process may influence this process and therefore post-hatch performance.

This study aims to put emphasis on the effects of *in ovo* administration of two different doses of L-carnitine at d 18 of incubation on hatching time and window and post-hatch performance of layer-type-chick.

MATERIALS AND METHODS

Experimental design: Hatching eggs from Hissex Brown layer breeders of 45 wk old provided by Grassett n.v. (Zulte, Belgium) were incubated with Petersime ® Vision 96 setter/hatcher at standard incubation conditions. In total, 9600 hatching eggs were set for incubation.

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At d 18 of incubation, a total of 600 eggs were available for the experiment. These eggs were divided in 4 groups of 150 eggs each i.e.:

- Control: eggs without any treatment
- Saline: eggs injected with saline solution (NaCl) of 0.9%
- LC500: eggs injected with L-carnitine of 500 μmole
- LC1000: eggs injected with L-carnitine of 1000 μmole

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. Between 490 h and 514 h of incubation, all the eggs were checked individually every 4 h for hatching. All unhatched eggs were opened to determine embryo development stage. At the end of hatch and within each group, day old chicks were weighed individually and assigned into 8 pens (2 pens per treatment) of 45 chicks each. Feed and water were provided *ad libitum*. For each pen, all chicks were weighed individually at 3, 7 and 14 d of age. Sample of 14 chicks were used to weigh residual yolk sac and intestine at 3 and 7 d of age. Intestine and residual yolk weights were used to determine their relative weights in function of chick body weights (BW). Numbers of chicks showing morbidity signs during the experiment were recorded.

Protocol of L-carnitine administration: At d 18 of incubation, 150 eggs of each group were injected with LC500, LC1000 or saline solutions. The remaining eggs were non-injected and served as the control. Injection of L-carnitine or saline solutions was made possible by candling each egg for evidence of living embryo and air chamber localization. Then, a needle 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. Eggs of saline group were injected with 100 μL of 0.9% saline solution. For L-carnitine administration, 78.99 μg or 157.98 μg was dissolved in 100 μL of 0.9% saline solution, respectively for LC500 and LC1000 groups. After injection into one of the holes, both holes were sealed with adhesive tape and the egg was placed in the hatching baskets.

Hatching: Between 490 and 514 h of incubation, the transferred eggs were checked individually every 4 h and the hatched chicks were recorded and weighed. For each egg, the incubation duration was defined as the time between setting and hatching. The spread of hatch was defined as the dispersion around the average incubation duration.

Morbidity definition: Morbidity was defined as chick showing sign of diarrhea in cloacal area. During the experiment, all the chicks were checked individually within each replication. For each group, numbers of chicks that showed sign of diarrhea were recorded. These numbers were used to calculate the proportions of chicks showing morbidity signs as:

$$\text{Morbidity} = \frac{100 \text{ x ni}}{\text{Ni}}$$

Where:

n = Number of chicks showing sign of diarrhea in the cloacal area
N = Total chick of chicks
i = Replication

Statistical analysis: The data were processed with a statistical software package of SYSTAT 11. The general linear models procedure was used to analyze chick, yolk sac and intestine weights and incubation duration in relation to treatments. Logistic regression was used to analyze the effect of treatments on morbidity and hatchability. For all analyses, P value of 0.05 was retained as the degree of significance.

RESULTS

Effect of *in ovo* injection of L-carnitine on incubation length and hatchability: Figure 1 shows hatching curve in function of incubation time and according to treatments. Overall, the beginning of hatching was delayed for at least 4 h in L-carnitine injected groups compared to control groups. With regard to number of hatched chicks, there were no significant differences between treatments at 490, 502, 510 and 514 h of incubation. However, between 494 h and 498 h of incubation, numbers of chicks of control and saline groups were higher than those of LC500 and LC1000 groups ($p<0.05$). But, at 506 h of incubation, numbers of hatched chicks in L-carnitine injected groups were higher compared to those of saline and control groups ($p<0.05$).

The spread of hatch (Fig. 1) was narrower for about 4 h in L-carnitine groups than in control and saline groups ($p<0.05$). Hatchabilities were not affected by *in ovo* L-carnitine administration and were 93% and 92%, respectively for control groups and L-carnitine groups.

Effect of *in ovo* injection of L-carnitine on chick weights, yolk sac and intestine weights: Day-old chick weights were similar between the four treatment groups. Overall, chick weight increased from 39.35 to 91.85 g during 14 d post-hatch. Figure 2 shows that average chick weights were comparable between treatments until d 14 of age.

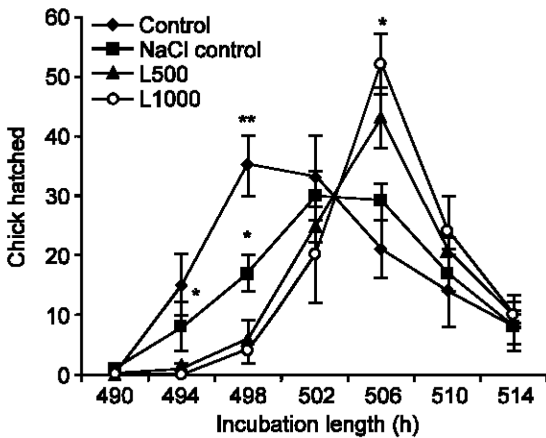

Fig. 1: Incubation length according to numbers of hatched chicks and treatments. For each incubation length, data sharing no common sign were different (p<0.05)

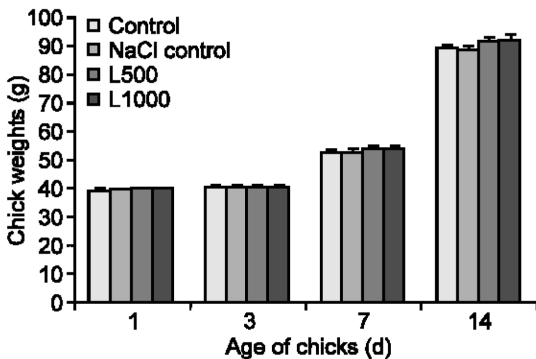

Fig. 2: Average (±SEM) chick weights (g) according to age and treatments. For each age, data sharing no common sign were different (p<0.05)

D-old chick yolk sac weights (data not shown) as well as yolk sac relative weight were comparable between treatments. Figure 3 indicates that at 3 and 7 d post-hatch, yolk sac relative weights were affected by treatments. At d 3, the lowest yolk sac relative weights were obtained in LC1000 group and the highest in saline group (p<0.01). At 7 days, yolk sac was significantly lower in the L-carnitine groups and almost reduced to zero while it was still about 2% of the 7 day chick weight in both control groups.

Chick relative intestine weights increased from 0.075 to 0.091 g/100 g, respectively at 3 and 14 d of age. There was no significant difference between the treatments (data not shown).

Effect of in ovo injection of L-carnitine on morbidity: Figure 4 shows percentages of morbidity signs according to treatments and in function of chick age. Percentage of chicks showing morbidity signs was

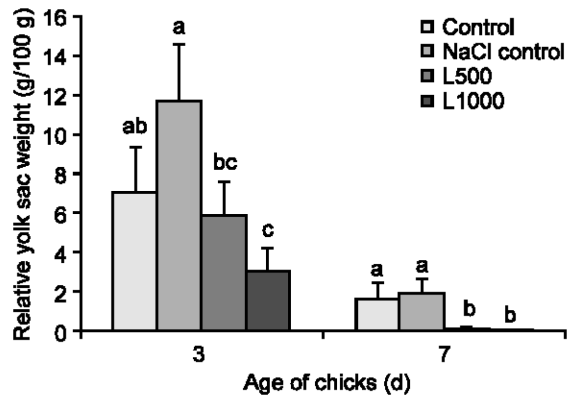

Fig. 3: Average (±SEM) relative yolk sac weights (g/100 g) according to the treatments (n = 14 chicks). Data sharing no common letter were different (p<0.05)

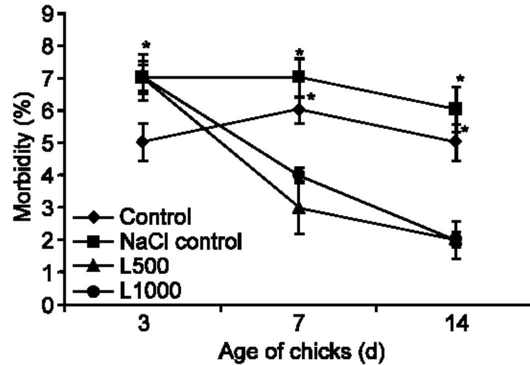

Fig. 4: Proportion of chicks showing morbidity signs (%) according to the treatments. Data sharing no common sign were different (p<0.01)

lower in control group than that of the three other groups at 3 d post-hatch (p<0.05). But, at d 7 and 14 of age, percentage of chicks with morbidity signs was lower in L-carnitine treated groups than those of saline and control groups (p<0.01).

DISCUSSION

The results of this study indicates clearly that *in ovo* administration of L-carnitine delayed hatching process with narrower hatching spread and faster utilization of yolk sac content during the first week post-hatch on one hand. On the other hand, increasing dose of L-carnitine leads to a decrease of percentage of chick with sign of morbidity during juvenile growth.

To our knowledge this is the first report about the effect of *in ovo* administration of L-carnitine on hatching process. Delayed hatching process in L-carnitine injected groups with narrower hatching spread may be partly explained by the time needed for L-carnitine to induce fat metabolism activity in the chick embryo,

followed by additional energy supply. Indeed, narrower spread of hatching in L-carnitine injected groups suggest that energy supply stimulates hatching process indicating that chicken embryo may need additional source of energy for efficient hatching process. Moreover, since the delayed in hatching process did not influence hatchability, it can be hypothesized that the timing of L-carnitine supplementation during embryonic life should occur earlier than d 18 of incubation. Earlier administration of L-carnitine may stimulate hatching process and as consequence may improve hatchability and chick quality. The lack of L-carnintine administration on hatchability reported in this study confirms the findings of Zhai *et al.* (2008). Moreover, Peebles *et al.* (2007) reported that no effect on hatchability of eggs from broiler breeders consuming 25 mg/kg of L-carnitine compared with their control counterparts. On contrary, the detrimental effect of injection of 1000 µmole of L-carnitine on hatchability and chick quality reported by Tona *et al.* (submitted) may be due to shorter incubation duration since incubation was stopped early at 504 h of incubation. Surprisingly, L-carnitine administration did not result in differences in absolute or relative yolk sac weight of d-old chick suggesting that unknown physiological factors can be involved in L-carnitine injection which may also influence hatching process.

Although there was a lack of effect of *in ovo* L-carnitine administration on yolk sac content utilization during embryonic life, yolk consumption increased with L-carnitine dose at d 3 and d 7 post-hatch indicating that its effect mainly acts in the first days of life post-hatch and that it lasts up to d 7 post-hatch.

Also, *in ovo* administration of L-carnitine did not lead to changes in body weight during the first 14 d post-hatch, indicating that yolk sac content utilization during juvenile growth is not necessarily related to changes in body weight as reported by Nouboukpo *et al.* (2010). It might influence more feed intake which is now under investigation in our laboratory. Interestingly, the positive correlation between the yolk sac relative weight and percentage of chicks with signs of morbidity observed in this study is in the line of the report of Nouboukpo *et al.* (2010). It can be hypothesized that *in ovo* injection of L-carnitine effect on yolk sac content utilization may provide enough energy to chicks in order to cope with new environmental conditions. Also, since yolk is the main site of immunoglobulins deposition, it can be suggested that reduced morbidity in L-carnitine treated groups may be due to early transfer of these immunoglobullins during juvenile life. However, mechanism by which *in ovo* injection of L-carnitine improved morbidity needs more investigation.

It is concluded that *in ovo* injection of 500 and 1000 µmol of L-carnitine into fertile Hissex Brown eggs at 18 d of incubation is not detrimental for hatchability, d-old chick weight and chick juvenile growth. Administration of L-carnitine during embryonic life advanced post-hatch yolk sac utilization and decreased percentage of chicks with sign of morbidity during post-hatch juvenile growth.

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