The effect of carnitine on hatching rate and metabolic profile of blood in breeding layers

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ABSTRACT: We examined the effect of orally administered L-carnitine (at a dose of 30 mg per kg of feeding mixture) on egg hatching and some haematological and biochemical parameters of blood plasma in COBB 500 breeding layers of meat type. The experimental results confirmed a positive effect of L-carnitine, which increased the hatching rate significantly ($P \leq 0.05$) by 8.89% as compared to the control. This positive effect resulted from a highly significant ($P \leq 0.01$) decrease in the number of unfertilized eggs in experimental layers (4.44%), as compared to the control (12.2%). Haematological tests such as total counts of erythrocytes and leukocytes showed no differences between control and experimental layers. However, the average levels of haematocrit and haemoglobin in experimental layers were significantly lower ($P \leq 0.01$) (0.31 l/l and 85.67 g/l) in comparison with the control (0.33 l/l and 89.92 g/l). Biochemical examination of the samples of blood plasma collected from experimental layers after the administration of L-carnitine revealed higher levels of glucose and magnesium and lower levels of total protein, cholesterol, AST, calcium and phosphorus, as compared to the control. The differences in the average levels of the monitored biochemical parameters between control and experimental layers were found highly significant ($P \leq 0.01$). The results provide new knowledge of the effect of L-carnitine on the metabolism of layers. These results are important not only from the scientific aspect, they are also of practical importance and can be used to formulate diets for breeding layers in order to enhance reproduction.

Keywords: layers; L-carnitine; reproduction; haematological and biochemical parameters

Discovered and described at the beginning of the last century, carnitine is still an attractive research topic of a number of experimental studies. Its major role with regard to beta-oxidation of fat was reported in 1957. The use of L-carnitine in clinical practice dates back to the 1980s and is associated with the possibility of industrial production of carnitine. L-carnitine can be found in a relatively wide range of human applications, for example medicine and dietetics, where it is widely used as a food additive. Recently, L-carnitine has been increasingly used as a supplement of feeding mixtures for both domestic and farm animals.

Although the animal organism is able to synthesise carnitine, carnitine deficiency may occur in animals exposed to stress during rearing or when high production and performance are required. Moreover, the ban on the use of animal-based meals that are the major exogenous source of carnitine has also contributed to carnitine deficiency. Efforts have therefore been made to supplement vegetable feeding mixtures with L-carnitine in order to increase the efficiency of farm animals and enhance fertility, physical performance and stress resistance of animals. Few scientific publications focus on the effect of L-carnitine on poultry reproduction. This subject was addressed for example by Leibetseder (1995), Rabie *et al.* (1997) and Neuman *et al.* (2002). It is assumed that carnitine may also have a positive effect on poultry reproduction, similar to that reported in different animal species by Baumgartner (1998) and Deana *et al.* (1989).

However, studies dealing with the effect of L-carnitine on the metabolic profile of poultry, particular-

ly on haematological and biochemical parameters of blood, are missing. Scientific literature provides the results of conventional haematological studies in layers that can only be used as basic data for initial comparison. This particularly concerns papers by Suchý *et al.* (1995, 2004), Straková *et al.* (2001), Večerek *et al.* (2003) and Tůmová *et al.* (2004), who studied the metabolic profile of layers during the reproductive period.

MATERIAL AND METHODS

The main aim of the present paper was to assess the effect of continuously administered feeding mixtures supplemented with L-carnitine on hatching rate and metabolic profile of blood in breeding layers.

The experiment was performed with a total of forty breeding layers and four roosters (meat-type COBB 500). The birds were divided into two groups (experimental group and control group). Each group consisted of twenty layers and two roosters. The animals were kept on deep bedding; watering and feeding was ensured by automatic watering devices and feeders. Layers aged 20 weeks were purchased from the company Mach Hatcheries in Litomyšl. Rearing proceeded in accordance with the technological guidelines "Breeder Management Guide COBB 500" provided by the Cobb Breeding Company. Both groups of layers were fed with a commercial feeding mixture (containing 893.08 g of dry matter, 165.68 g of proteins, 7.87 g of lysine, 3.65 g of methionine, 6.61 g of sulphur-containing amino acids, 5.66 g of threonine, 1.93 g of tryptophan, 9.91 g of arginine, 28.29 g of fat, 26.41 g of fibre, 11.63 MJ of ME, 109.15 g of ash, 29.53 g of Ca, 6.81 g of P, 1.58 g of Mg, 126.31 mg of Fe, 15.52 mg of Cu, 148.87 mg of Mn, 136.42 mg of Zn, 0.43 mg of Se, 15 000 i.u. of vitamin A, 3 000 i.u. of vitamin D_3, and 80 mg vitamin E per 1 kg of feeding mixture). L-carnitine was added into the experimental feeding mixture at a dose of 30 mg/kg. The experiment was started with layers aged 28 weeks; the layers were examined regularly every four weeks (i.e. at the age of 28, 32, 36, 40, 44, 48, 52, 56, and 60 weeks). The examinations included weighing, haematological and biochemical tests, counting the number of laid eggs followed by placing the collected eggs in an incubator.

The number of eggs laid by layers aged 28 to 60 weeks during the experiment was monitored in order to calculate the laying intensity. Eggs were collected one week before hatching; 30 eggs from the control layers and 30 eggs from the experimental layers were selected and placed in an incubator. The eggs placed in the incubator were selected on the basis of the following criteria: weight ranging between 60 g and 70 g, intact shell and the absence of other visual morphological defects on the shell. The incubator OCTAGON 100 was used for hatching. Incubation proceeded exactly according to the manufacturer's technological instructions for use supplied by the manufacturer of this incubator. The number of both hatched and unhatched eggs was evaluated after the incubation and egg hatching had been completed. In the case of unhatched eggs, the number of unfertilized eggs and eggs containing dead foetuses was determined. The total of nine hatching cycles (including 270 eggs) in each group were carried out.

Blood samples for haematological and biochemical examination were collected from 10 layers selected from each group at 7 a.m. every four weeks, i.e. the total of 90 examinations were performed during the experimental monitoring in both control and experimental layers. Blood for examination obtained by the puncture of *vena basilica* was stabilized with heparin. Biochemical parameters were determined in blood plasma obtained after centrifuging heparin-stabilized blood at 3 000 rpm for 30 minutes.

The following haematological parameters were determined: total erythrocyte count (Er), haemoglobin level (Hb), haematocrit level (Hk). Total erythrocyte count was determined by using a flask dilution method and counting the number of red blood cells using the Bürker chamber by means of the Natt-Herrick diluent. The level of haemoglobin was determined photometrically at a wavelength of 540 nm. The assay for haematocrit level was performed in special microhaematocrit capillaries after centrifuging the horizontally positioned capillaries in the Janetzki centrifuge at 16 000 revolutions per minute for 3 minutes.

Total leukocyte count was determined in an analogous manner as total erythrocyte count, i.e. by using a flask dilution method and counting the number of white blood cells using the Bürker chamber by means of the Natt-Herrick diluent.

The blood plasma of layers was also analysed for the following organic substances: total plasma protein (CB), glucose (Glu), cholesterol (Chol), catalytic concentrations of AST and ALT. The following mineral substances were determined in blood plasma:

calcium (Ca), phosphorus (P) and magnesium (Mg). The above-mentioned biochemical parameters of blood plasma were determined photometrically by commercially available testing kits Bio-La-Test (Pliva-Lachema Brno).

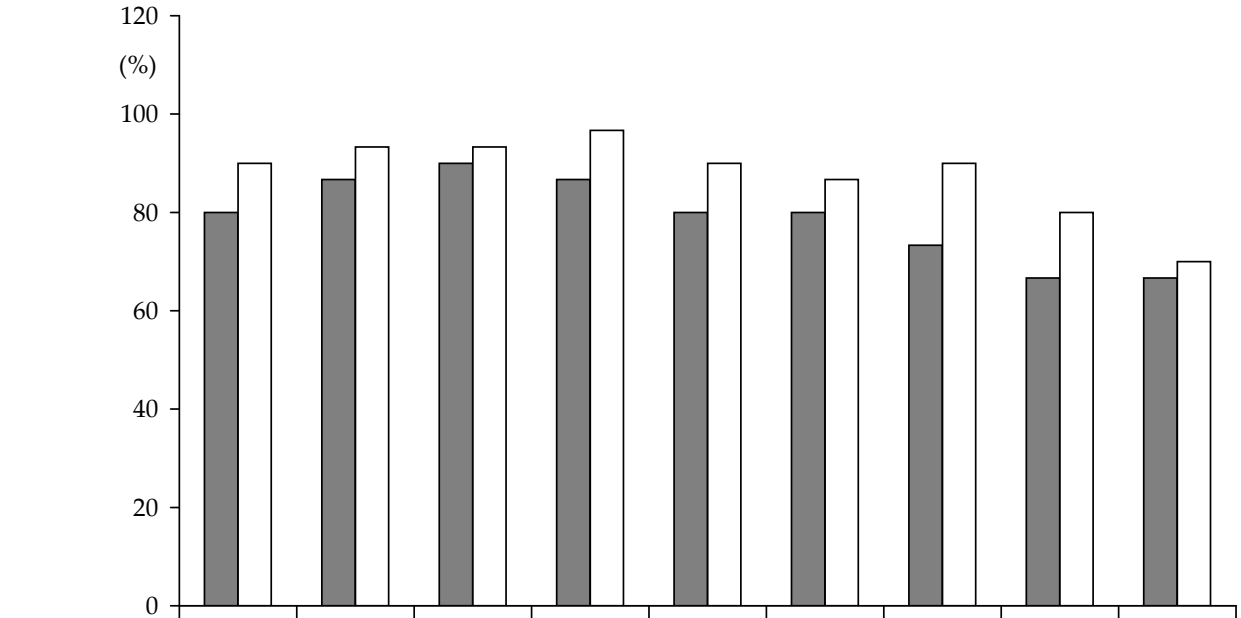
The results were processed by mathematical and statistical methods; arithmetic mean ($\bar{x}$), standard deviation (s_n), standard error of the arithmetic mean ($s_{\bar{x}}$) and coefficient of variation (v) were calculated. In order to assess the significance of differences in average levels, Student's test with the probability factors of $P \leq 0.05$ (*) and $P \leq 0.01$ (**) was employed. The mathematical and statistical processing of the results was performed by the programme Statgraphics.

RESULTS

During the experimental period the weighing of layers and the collection of blood samples were performed at the same intervals. The weight of layers increased gradually during the experiment (from the 28th to the 60th week of age); the weight of control and experimental layers increased from 2.98 kg to 3.88 kg and from 2.90 kg to 3.75 kg, respectively. The differences in average live weight between control and experimental layers were statistically insignificant during the experimental monitoring.

The laying intensity was examined from the production aspect. The laying intensity of layers aged 28–36 weeks gradually increased from 77.86% to 83.57% in the control group and from 75.11% to 85.00% in the experimental group. After the 40th week of age, the laying intensity decreased from 78.57% to 62.70% in the control group and from 76.43% to 66.43% in the experimental group. No statistically significant differences in the laying intensity were found between both groups.

The total of 9 hatching cycles were performed in four-week intervals with layers between the 28th and the 60th week of age; the course of hatching is illustrated in Figure 1. The hatching rates in the control and experimental group were 78.89% and 87.78%, respectively. Of the total number of incubated eggs, 21.11% did not hatch in the control group and 12.59% in the experimental group. The difference between these two average values was significant ($P \leq 0.05$). The analysis of unhatched eggs in the control group showed that 12.22% of unhatched eggs were unfertilized and 8.89% of unhatched eggs contained dead foetuses. In the experimental group, 4.44% of unhatched eggs were unfertilized and 8.15% of unhatched eggs contained dead foetuses.



Weeks	1	2	3	4	5	6	7	8	9
Control	80.00	86.67	90.00	86.67	80.00	80.00	73.33	66.67	66.67
Experiment	90.00	93.33	93.33	96.67	90.00	86.67	90.00	80.00	70.00

Figure 1. The results of hatching

The difference in the average level of unfertilized eggs between the two groups (i.e. 12.22% in the control group and 4.44% in the experimental group) was highly significant ($P \leq 0.01$).

The experiment also included regular haematological and biochemical examinations. According to the results of haematological tests, the two groups of layers did not differ in cellular elements such as total erythrocyte count and total leukocyte count. The erythrocyte count in control layers during the monitored period ranged from 1.72 T/l to 3.81 T/l, while experimental layers it showed the range of 1.73 T/l–3.77 T/l. The total leukocyte count ranged from 12.10 G/l to 21.80 G/l in the control group and from 11.80 G/l to 19.94 G/l in experimental layers. Control layers showed the average erythrocyte count of 2.35 T/l and the average leukocyte count of 14.48 G/l while experimental layers showed the average erythrocyte count of 2.33 T/l and the average leukocyte count of 13.92 G/l (Table 1).

Table 1. The results of haematological and biochemical examinations of blood plasma in the COBB breeding layers

Parameter	Gr	n	$\bar{x}$	s_n	$s_{\bar{x}}$	v	P
Er (T/l)	C	90	2.35	0.331	0.035	14.06	–
	E	90	2.33	0.319	0.034	13.69	0.524
Hc (l/l)	C	90	0.33	0.025	0.003	7.42	**
	E	90	0.31	0.042	0.004	13.39	4.136
Hb (g/l)	C	90	89.92	10.106	1.065	11.24	**
	E	90	85.67	10.315	1.087	12.04	2.793
Le (G/l)	C	90	14.48	3.994	0.421	27.59	–
	E	90	13.92	3.36	0.383	26.12	0.978
TP (g/l)	C	90	54.03	5.316	0.560	9.84	**
	E	90	50.55	5.301	0.559	10.49	4.398
Gluc (mmol/l)	C	90	13.70	1.207	0.127	8.81	*
	E	90	14.07	0.868	0.092	6.17	2.389
Chol (mmol/l)	C	90	4.16	1.019	0.107	24.50	**
	E	90	3.25	0.706	0.074	21.73	6.965
AST (μcat/l)	C	90	1.079	0.125	0.013	13.33	**
	E	90	0.992	0.101	0.011	16.56	5.120
ALT (μcat/l)	C	90	0.047	0.025	0.003	37.33	–
	E	90	0.047	0.016	0.002	34.20	0.000
Ca (mmol/l)	C	90	6.12	0.828	0.087	13.52	**
	E	90	5.54	1.245	0.131	22.48	3.716
P (mmol/l)	C	90	1.77	0.281	0.030	15.83	**
	E	90	1.63	0.267	0.028	16.39	3.567
Mg (mmol/l)	C	90	0.82	0.047	0.005	5.79	**
	E	90	0.85	0.053	0.006	6.24	3.416

Gr – group, C – control layers, E – experimental layers, Er – total erythrocyte count, Hc – haematocrit value, Hb – content of haemoglobin, Le – total leukocyte count, TP – total protein, Gluc – glucose, Chol – cholesterol, AST and ALT – catalytic concentrations of transaminases, Ca – calcium, P – phosphorus, Mg – magnesium, n – number of measurements, $\bar{x}$ – arithmetic mean, s_n – standard deviation, $s_{\bar{x}}$ – standard error of the arithmetic mean, v – coefficient of variation, P – significance

*$P \leq 0.05$; **$P \leq 0.01$

Highly significant differences between both groups were found ($P \leq 0.01$) in the case of haematocrit and haemoglobin; lower average levels were found in the experimental group (Table 1). Haematocrit levels ranged between 0.28–0.41 l/l (control) and 0.24–0.36 l/l (experiment) during the experimental period. The levels of haemoglobin in control and experimental layers during the monitored period ranged between 78.48 and 101.60 g/l and between 64.42 and 99.85 g/l, respectively.

The administration of L-carnitine to experimental layers also induced significant changes in the monitored biochemical parameters (Table 1). A significant increase ($P \leq 0.05$) in plasma glucose in experimental layers (14.07 mmol/l) was detected, as compared to the control (13.70 mmol/l), and a highly significant increase ($P \leq 0.01$) of plasma magnesium (0.85 mmol/l) in experimental layers, as compared to the control (0.82 mmol/l).

The average levels of the following parameters in blood plasma were very significantly ($P \leq 0.01$) lower in experimental layers as compared to the control: total protein 50.55 g/kg (control: 54.03 g/kg), cholesterol 3.25 mmol/l (control: 4.16 mmol/l), AST 0.992 μcat/l (control: 1.079 μcat/l), calcium 5.54 mmol/l (control: 6.12 mmol/l) and phosphorus 1.63 mmol/l (control: 1.77 mmol/l). The catalytic concentration of ALT was the only biochemical parameter that showed the same average level in both groups, i.e. 0.047 μcat/l. The catalytic concentrations of ALT in blood plasma during the experimental period ranged between 0.030 and 0.055 μcat/l in the control group and between 0.028 and 0.060 μcat/l in the experimental group.

During the experimental period, i.e. in layers between the 28th and the 60th week of age, the level of the selected biochemical parameters in control and experimental layers ranged as follows: total protein – 43.63–66.94 g/l and 39.53–58.56 g/l, glucose – 12.51–15.74 mmol/l and 12.68–15.03 mmol/l, cholesterol – 2.43–6.44 mmol/l and 1.46–4.07 mmol/l, AST – 0.94–1.64 μcat/l and 0.93–1.05 μcat/l, ALT – 0.030–0.055 μcat/l and 0.028–0.060 μcat/l, calcium – 5.19–6.77 mmol/l and 4.59–6.55 mmol/l, phosphorus – 1.38–2.14 mmol/l and 1.33–1.85 mmol/l, and magnesium – 0.78–0.89 mmol/l and 0.79–0.89 mmol/l.

DISCUSSION

This experiment confirmed a positive effect of feeding mixtures supplemented with L-carnitine (fed to breeding layers) on the hatching rate. Moreover, this experiment also showed that the live weight of layers and the laying intensity were not significantly affected. A positive finding is that the hatching rate of eggs laid by experimental layers increased by 8.89%. The analysis of the number of unhatched eggs revealed that a higher hatching rate was attributed to a higher rate of egg fertilization. The number of unfertilized eggs related to the total number of incubated eggs in the control group was higher (12.22%) than in the experimental group (4.44%) while no difference between both groups was found in the relative number of eggs containing dead foetuses (8.15% in the control and 8.89% in the experimental group). The results obtained are in good agreement with the conclusions drawn by Rabie *et al.* (1997), who found that L-carnitine supplementation did not significantly affect egg production. The hatching rate increased significantly, which is also supported by the findings reported by Leibetseder (1995). We assume that the increased rate of hatching results from the action of L-carnitine on the metabolism of both layers and breeding roosters which were fed with the same feeding mixture. This assumption is also confirmed by the findings of Neuman *et al.* (2002), who reported the increased levels of sperm cells in breeding roosters that were administered L-carnitine. Our studies that have not been published so far revealed increased activity of sperm cells in breeding roosters.

On the basis of haematological and biochemical analyses it can be concluded that the long-term continuous supplementation of a diet with L-carnitine induces some changes in the metabolic profile of layers.

Haematological examinations showed that L-carnitine administered to experimental layers did not affect the total counts of erythrocytes and leukocytes as seen by comparison with control layers. However, haematocrit and haemoglobin levels in experimental layers were significantly lower ($P \leq 0.01$), as compared with the control.

Although the levels of these haematological parameters in experimental layers decreased, they still fell in the physiological ranges reported by Jeřábek *et al.* (1993), Straková *et al.* (2001), Večerek *et al.* (2003), Suchý *et al*. (2004) and Tůmová *et al.* (2004) for clinically healthy layers during a laying period.

In the case of biochemical parameters, experimental layers showed ($P \leq 0.01$) increased levels of plasma glucose which may indicate a positive effect

of L-carnitine on the energetic metabolism of layers. Furthermore, the levels of plasma magnesium in experimental layers were significantly higher ($P \leq 0.01$) compared with the control.

The continuous oral administration of L-carnitine to experimental layers resulted in the significant ($P \leq 0.01$) decrease in molar concentrations of total protein, cholesterol, calcium, and phosphorus including the catalytic concentration of AST. We assume that the decreased levels of biochemical parameters might be associated with the increasing utilization of nutrients, i.e. with the increasing rate of nutrient metabolization. One positive finding is that the catalytic concentration of AST decreased. In this respect, L-carnitine can be considered as a protective agent protecting particularly the liver parenchyma.

As for haematological results, the results of assays for the selected biochemical parameters also fell in the respective physiological ranges although there were some differences between control and experimental layers. This finding is also in good agreement with the results reported by Suchý *et al.* (1995, 1999, 2001) for layers during a laying period.

It follows from the results that the continuous oral administration of L-carnitine induces changes in the intermediary metabolism of layers, improving reproductive parameters and particularly increasing the hatching rate. On the basis of this conclusion, it can be recommended that feeding mixtures be supplemented with L-carnitine. Carnitine supplementation should particularly be applied to the plant-based feeding mixtures for breeding poultry.

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ABSTRAKT

Vliv podávání karnitinu na líhnivost a metabolický profil krve plemenných nosnic

Práce se zabývá vlivem kontinuálního perorálního podávání L-karnitinu, v dávce 30 mg/kg krmné směsi, na líhnivost vajec a vybrané hematologické a biochemické ukazatele krevní plazmy plemenných nosnic masného typu COBB 500. Výsledky experimentu potvrdily pozitivní vliv L-karnitinu v podobě průkazně ($P \leq 0,05$) zvýšené líhnivosti o 8,89 %

oproti kontrolní skupině. Z výsledků vyplývá, že tento pozitivní efekt byl způsoben vysoce průkazným ($P \leq 0,01$) snížením počtu neoplozených vajec u pokusných nosnic (4.44 %) oproti kontrolním (12.2 %). Výsledky hematologických vyšetření u celkového počtu erytrocytů i leukocytů neprokázaly rozdíly mezi kontrolními a pokusnými nosnicemi. U hematokritové hodnoty a u obsahu hemoglobinu byly u pokusných nosnic stanoveny vysoce průkazně ($P \leq 0,01$) nižší průměrné hodnoty (0,31 l/l a 85,67 g/l) oproti kontrole (0,33 l/l a 89,92 g/l). Závěry biochemických vyšetření dokládají, že po podávání L-karnitinu vykazovaly nosnice v krevní plazmě vyšší průměrné hodnoty glukózy, hořčíku a nižší průměrné hodnoty celkové bílkoviny, cholesterolu, AST, vápníku a fosforu oproti kontrolním nosnicím. Rozdíly mezi průměrnými hodnotami u sledovaných biochemických ukazatelů u kontrolní a pokusné skupiny nosnic byly testovány jako vysoce průkazné ($P \leq 0,01$). Dosažené výsledky představují nejen vědecký přínos v podobě nových poznatků o vlivu L-karnitinu na metabolismus nosnic, ale i praktický přínos, který lze využít ve výživě plemenných nosnic ke zlepšení reprodukce.

Klíčová slova: nosnice; L-karnitin; reprodukce; hematologické a biochemické ukazatele

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