

## **Metabolism and productivity**

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### **EFFECTS OF DIFFERENT LEVELS OF PROTECTED L-CARNITINE ON MILK PRODUCTION, METABOLISM, AND REPRODUCTIVE PARAMETERS OF HIGH-PERFORMANCE DAIRY COWS**

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#### **Abstract**

Amino acid and vitamin provision is known to be crucially vital in the nutrition of highly productive dairy cattle. Currently, important role of carnitine in carbohydrate, fat and protein metabolism has been established, including the transport of long-chain fatty acids through the mitochondrial membrane, excretion of potentially toxic metabolites from cells, regulation of gluconeogenesis, and synthesis of growth hormone. However, there are no recommendations on dietary L-carnitine dosage for newly calved cows with different milk yields. Moreover, data on the effect of dietary carnitine are often contradictory. In this paper, we for the first time compare effective postpartum doses of Carnipass™ and determine optimal amount of protected of L-carnitine (45 mg per 1 kg of produced milk). The aim of our research was to study the effect of different doses of dietary L-carnitine in a protected form on milk productivity, quality, intensity and peculiarities of physiological and biochemical processes in cows during early post calving period. Experiments, including physiological ones, were carried out on four groups of Holstein black-motley cows (8 cows per each, Dubrovitsy unit of Klenovo-Chegodaevo experimental farm, Moscow Province, 2014). Farm tests (Agrofirma Detchinskaya, Maloyaroslavetsky Redion, Kaluga Province) was conducted in 2015-2016. All animals ate a basic ration. The cows of experimental groups II, III and IV additionally received dietary Carnipass™ (Lohmann Animal Health GmbH & Co. KG, Germany), 5.5, 8.3 and 11.1 g equal to 1.0, 1.5 and 2.0 g of L-carnitine, respectively, 21 days before calving and 30, 45 and 60 mg of L-carnitine per 1 kg of produced milk for 120 days after calving. The average daily milk yield in cows of groups II, III and IV was 29.5, 31.6 and 31.4 kg, or by 0.9, 3.0 ( $p < 0.05$ ) and 2.8 kg ( $p < 0.05$ ) higher compared to the control group. When recalculated for milk with 4 % fat content, this index was 3.7, 10.7 ( $p < 0.01$ ) and 10.0 % higher ( $p < 0.01$ ). The somatic cell counts in the milk of experimental cows were from  $217.5 \times 10^3$  to  $42.0 \times 10^3$  per 1 cm<sup>3</sup>, which is  $50.0-74.5 \times 10^3/\text{cm}^3$  lower than in the control group. The use of L-carnitine at different doses allows reduction of costs per 1 kg of 4 %-fat milk by 3.8-10.8 % feed energy units, 3.6-10.5 % digestible protein, and 3.8-10.7 % concentrated feed. Dietary L-carnitine (45 and 60 mg/kg of milk) increases digestibility of dry matter by 3.06-2.71 %, of proteins by 3.79 ( $p < 0.05$ ) and 3.90 % ( $p < 0.05$ ), respectively, of fat by 0.55-0.06 %, of cellulose by 2.13-1.49 %, and of nitrogen-free extractive substances by 3.41-2.66 %. In cows fed with L-carnitine, blood protein reserves increase due both to albumins and globulins, and protein index is 5.4, 17.0 ( $p < 0.05$ ) and 15.1 % ( $p < 0.05$ ) higher. Activity of transamination enzymes, alanine aminotransferase and aspartate aminotransferase, also rises. The blood concentration of urea decreases by 30.1-35.5 %, which also indicates better nitrogen metabolism. Increase in glucose content by 4.7-9.8 %, and decrease in the amount of bilirubin and cholesterol by 1.4-4.9 and 3.9-8.8 %, respectively, may indicate better function of the liver. At a dosage of 45 mg/kg L-carnitine improves parameters of non-specific immunity. Phagocytic index, bactericidal and lysozyme activity increase by 0.85 units ( $p < 0.01$ ), 6.91 % ( $p < 0.001$ ) and 5.43 % ( $p < 0.001$ ), respectively. In commercial farm tests (Detchinskaya Agro-Firm, Maloyaroslavetskii Region, Kaluga Province, 2015-2016) L-carnitine in a dose of 45 mg/kg has increased milk yield by 11.6 % ( $p < 0.05$ ), as recalculated for 4 %-fat milk, with a decrease in costs per unit of milk production. This is in line with our experimental data. Insemination index is also 0.5 units less, and the service period is 24 days shorter. The profit due to use of protected dietary L-carnitine (45 mg/kg) was 3808 rubles per cow.

Keywords: high producing dairy cows, feed additives, L-carnitine, digestion, digestibility, metabolism, immunity, milk production, reproduction

Along with optimization of feeding [1] and improvement of diets [2] it is reasonable to use methods promoting improved functioning of gastrointestinal tract, liver, and other systems contributing to more effective metabolism in highly productive cows [3] to use their productivity potential [4].

It is known that one of the most important components of biologically complete nutrition of animals and humans is amino acid supply [5, 6]. Important role of methylation in genome functioning [7], immune processes, stress responses and physical loads [8], body detoxication [9] due to lipotropic-hepatoprotective action of methyl-containing compounds [10, 11] had been proven. Acute deficit of methyl-containing metabolites in highly productive dairy cattle is due to their insufficient level in the feed [12, 13]. Even when diets are enriched with high-protective additives (soybean, rape, and sunflower cakes), the diets require additional sources of methyl-containing compounds in form of methionine, the first limiting amino acid for ruminants [14, 15], as well as choline, betaine, vitamins and mineral substances participating in synthesis of vital compounds for transmethylation [16].

Recently, particular attention is paid to carnitine, which is synthesized in animal body at sufficient supply of lysine, methionine, choline, vitamins C, B<sub>3</sub>, B<sub>9</sub>, B<sub>12</sub>, B<sub>15</sub>, cobalt, and iron [17, 18]. Carnitine plays important role in carbohydrate and protein metabolism [19, 20]. Carnitine participates in transport of long-chain fatty acids through mitochondrial membranes [21], in removing potentially toxic metabolites from cells, in regulation of gluconeogenesis [22], in synthesis of growth hormone [23, 24]. L-carnitine is used in medicine to maintain cardio-vascular function [25], to strengthen heart muscle and control blood cholesterol level, to render neuroprotective effect, to improve immunity, to prevent atherosclerosis and heart attack, to promote body restoration after loads and stresses [26], to regenerate tissues, to grow muscle tissue more intensively (anabolic action) [27, 28]. L-carnitine inhibits accumulation of arachidonic acid in phospholipids of thrombocytes [29], participates in body detoxication [30], is widely used for treatment of kidney and liver diseases [31], acute infectious diseases, during pregnancy and lactation [32, 33], and for improvement of sperm production in agricultural animals [34, 35].

Post-calving period in highly productive cows is associated with significant reconstruction and acceleration of metabolic processes [36, 37]. Herewith, tissue lipoproteins are used to ensure milk synthesis, which results in a reduction of body weight in newly-calved cows, incidence of ketosis, hepatitis and other diseases [38], significant milk deficit, decrease in productive use, and deterioration of reproductive function [7].

Although outcomes of numerous researches evidence on the effective use of protected L-carnitine in diets of cattle, recommendations on L-carnitine dosages for newly-calved cows with dissimilar productive capacity are missing. Besides, information on the effect of additionally supplied carnitine are often contradictory [39] and require further detailed studying of physiological and productive action of various dosages of protected L-carnitine in cattle feeding.

Present paper introduces different dosages of protective L-carnitine (in form of Carnipass™) in diets of newly-calved highly productive cows in terms of its influence on digestibility and use of nutritive feed substances, ruminal metabolism, morphological, biochemical, and immunological blood parameters, milk yield and milk quality values. Finally, the optimal dosage of protected L-carnitine (45 mg per 1 kg of produced milk) was established and recommended for use.

Our purpose was to study the effect of various dosages of protected L-

carnitine on milk yield, qualitative milk values, intensity and character of physiological and biochemical processes in newly-calved highly productive cows during days in milk (DIM).

*Techniques.* For studies (Dubrovitsy farm, research farm Klenovo-Chegodaevo, Dubrovitsy Village, Moscow Region, 2014), four groups of pure-breed Black Pied Holstein cows (*Bos taurus taurus*) of the 2<sup>nd</sup> and 3<sup>rd</sup> lactations (8 animals per group) were formed accounting for number of calvings, body weight, and milk yield during previous lactation period. Experimental animals in group I (control) and tested groups II-IV got main diet prepared according to the standards [40]. In addition to feed, 21 days in advance to calving the animals from groups II, III and IV got 5.5; 8.3 and 11.1 g of Carnipass™ (Lohmann Animal Health GmbH & Co. KG, Germany) with 18 % of active substance, or 1.0; 1.5 and 2.0 g of protected L-carnitine as per active substance. After calving, cows got 30; 45 and 60 mg of protected L-carnitine per 1 kg of produced milk within 120 days.

Feed consumption was determined by the difference between the supplied quantity and the residual quantity. Milk samples were taken from each cow each ten days. Each average daily sample for analysis was proportional to the produced milk (according to the State Standard GOST 13928-84). Quantitative composition of protein, fat, milk sugar, Ca, P in samples was assessed with an analyzer Bentley 150 (Bentley Instruments, Inc., USA) according to GOST 5867-90, GOST 25179-90, GOST 3626-73, GOST 3625-84, and GOST 3624-92. Milk fatness was determined butyrometrically by Gerber method, protein by formol titration, dry substance by calculation, number of somatic cells according to GOST 23453-90. Productive performance of each cow during the experiment, as well as in average by a group of animals was estimated both as volume of milk of natural fatness and as that re-calculated in terms of 4 %-fat milk. Feed use (energetic feed units, digested protein and concentrates) were determined based on actual consumption related to 1 kg of 4%-fat milk.

Physiological digestion trial to study the digestibility of nutritional substances was conducted in animals of I, III and IV groups 90 days after calving (3 cows with average productive capacity were taken from each group). During the digestion trial the cows were in individual standing stalls equipped by feedboxes and fecal and urine collection facilities. Accounting period took 5 days. Average daily samples of feed, their residues, fecal masses and urine were collected and analyzed by common zootechnical methods [41].

At the end of test, concentration of total protein and its fractions, concentration of glucose, urea, alanine aminotransferase (ALT), aspartate aminotransferase (AST), bilirubin, alkaline phosphatase, cholesterol, calcium, and phosphorus was determined 3 hours after feeding in blood samples taken from the animals of each group ( $n = 3$ ) with the use of an automatic biochemical analyzer Chem Well (Awareness Technology, Inc., USA). Bacterial activity was assessed by photo nephelometric method, lysozyme activity by V.I. Mutovin [42], phagocyte activity by the ability of blood cells to ingest and digest.

To study ruminal metabolism, ruminal digesta was taken from highly productive cows 3 hours after feeding with further determination of pH, content of volatile fatty acids (VFA), ammonium nitrogen, mass fractions of protozoon and bacteria. Acidity was determined with a pH-meter Aquilon-410 (Aquilon Corp., Russia), total number volatile fatty acids by steam distillation with a Markham apparatus, ammonium nitrogen by Conway's micro-diffusion method, total nitrogen by Kjeldahl method after trichloroacetic acid-induced precipitation [43]. Biomass of protozoon and bacteria was determined by differentiated centrifugation.

In pursuance of testing, two groups of Red Pied cows, 25 cows each,

were formed (LLC Agrofirma Detchinskoye, Maloyaroslavetskiy District, Kaluga Region, 2015–2016). Cows in the tested group were fed with protected carnitine 21 days in advance of calving (dosage  $8.3 \text{ g} \cdot \text{animal}^{-1} \cdot \text{day}^{-1}$ ) and on day 120 after calving (45 mg per 1 kg of produced milk) with combined feed in addition to the main balanced common diet. Animals in control group were not supplied with carnitine.

Milk yield, qualitative milk properties, and feed costs were assessed by the above described methods. Animals were weighted during morning hours before feeding in different physiological periods: before launching, 3–5 days before calving, and 5 days, 1, 2, 3, 4 months after calving. Insemination index and service period was established. Based on zootechnical records, economic feasibility of supply of L-carnitine in alimentation of highly productive dairy cattle was estimated during testing.

Data was biometrically processed by *t*-Student criteria. Average mean (*M*), standard error of mean ( $\pm$ SEM), and statistical significance (*p*) values were calculated. Test results were considered highly statistically significant at  $p < 0.001$ , and statistically significant at  $p < 0.01$  and  $p < 0.05$  (44).

**Results.** Consumption of nutritional substances and energy by cows in tested groups getting different dosages of protected L-carnitine was comparatively equal and ensured high milk yields (Table 1).

In adding protected L-carnitine to the diet (30; 45, and 60 mg/kg of produced milk), average daily milk yields in cows of groups II, III, and IV was 29.5; 31.6, and 31.4 kg, i.e. exceeded the control by 0.9; 3.0 ( $p < 0.05$ ) and 2.8 kg ( $p < 0.05$ ). In terms of 4 %-fat milk, the difference was 3.7; 10.7 ( $p < 0.01$ ) and 10.0 % ( $p < 0.01$ ), respectively. Milk fat, protein, and lactose levels in daily milk of cows of tested groups were 3.6–10.7; 3.4–11.0, and 4.0–10.7 % higher as compared to control. Number of somatic cells in milk produced from cows in tested groups averaged 217.5–242.0 ths/cm<sup>3</sup> and was lower than in the control by 50.0–74.5 ths/cm<sup>3</sup>, not exceeding the norms for the highest grade. Content of dry substance, fat, protein, lactose, calcium, and phosphorus in milk of cows in the control and tested groups was relatively equal. Use of different dosages of protected L-carnitine promoted lowering of feed costs: by 3.8–10.8 % for energy feed units, by 3.6–10.5 % for digested protein, by 3.8–10.7 % (per production of 1 kg of 4 %-fat milk) for concentrates as compared to control.

### 1. Milk yield and quality in Black Pied Holstein cows receiving dietary Carnipass™ during the first 4 months of lactation (*M*±SEM, Dubrovitsy Farm, Experimental Farm Klenovo-Chegodaevo, 2014)

Indicator	Group			
	I (control) ( <i>n</i> = 8)	II ( <i>n</i> = 8)	III ( <i>n</i> = 8)	IV ( <i>n</i> = 8)
Quantitative indicators				
Natural milk, kg	28.60±0.63	29.50±0.81	31.60±0.72*	31.40±0.73*
Fat in milk, %	4.20±0.19	4.22±0.21	4.21±0.17	4.21±0.17
4 % milk, kg	30±0.47	31.1±0.53	33.2±0.65**	33.0±0.64**
Total volume of natural milk, kg	3432	3540	3792	3768
Total volume of 4%-fat milk, kg	3600	3732	3984	3960
Milk quality indicators				
Dry substance, %	14.12±0.21	14.16±0.24	14.19±0.27	14.20±0.31
Protein, %	3.30±0.36	3.31±0.27	3.32±0.19	3.31±0.34
Lactose, %	4.82±0.03	4.82±0.02	4.82±0.03	4.82±0.02
Somatic cells, ths/cm <sup>3</sup>	292.0±70.5	242.0±67.7	217.5±65.4	225.0±64.6
Calcium, %	0.163±0.01	0.162±0.01	0.163±0.01	0.163±0.01
Phosphorus, %	0.950±0.004	0.960±0.005	0.950±0.004	0.950±0.004

Note. See description of groups in *Techniques* section. Test period was 120 days.

\*, \*\* Differences from control are statistically significant at  $p < 0.05$  and  $p < 0.01$ , respectively.

Upon feeding of highly productive newly-calved cows with dietary pro-

tected L-carnitine at 45 and 60 mg/kg milk dosages, dry substance digestibility increased by 3.06-2.71 %, protein by 3.90-3.79 %, fat by 0.55-0.06 %, fibre by 2.13-1.49 %, nitrogen free extractive substances by 3.41-2.66 % as compared to control (Table 2). Carnitine promoted better use of nitrogen by 3.1-3.3 g as compared to control and ensured valid differences in protein digestibility ( $p < 0.05$ ).

## 2. Feed nutrient digestibility coefficients (%) in Black Pied Holstein cows receiving dietary Carnipass™ ( $M \pm SEM$ , Dubrovitsy Farm, Experimental Farm Klenovo-Chegodaevo, 2014)

Indicator	Group		
	I (control, $n = 3$ )	III ( $n = 3$ )	IV ( $n = 3$ )
Dry substance	72.20 $\pm$ 1.24	75.30 $\pm$ 1.18	75.00 $\pm$ 1.19
Organic substance	74.60 $\pm$ 1.16	77.60 $\pm$ 1.19	77.20 $\pm$ 1.23
Protein	70.10 $\pm$ 1.06	74.10 $\pm$ 0.12*	73.90 $\pm$ 1.14*
Fat	70.30 $\pm$ 1.09	70.90 $\pm$ 1.27	70.40 $\pm$ 1.23
Fibre	61.80 $\pm$ 1.21	64.90 $\pm$ 1.21	63.30 $\pm$ 1.35
Nitrogen free extractives	74.50 $\pm$ 1.14	77.90 $\pm$ 1.34	77.10 $\pm$ 1.37

Note. See description of groups in *Techniques* section. Test period was 120 days.

\* Differences from control are statistically significant at  $p < 0.05$ .

In general, ruminal pH was within 6.65-6.79. In groups II, III and IV, concentration of ammonia was 17.56; 18.92 and 18.98 mg% (0.37; 1.36 and 1.79 mg% higher than in control), of volatile fat acids 9.34; 11.22 and 11.27  $\mu$ mol/100 ml (0.21; 2.09 and 2.14 % higher than in control). Growth in levels of ruminal metabolites was due to acceleration of microbial processes in forestomach and growth in the number of protozoon in rumen (by 6.7-36.6 %), and of bacteria (by 9.1-31.8 %) as compared to the control values. Such significant changes in microbial processes in the intestinal tract promoting better feed digestion were, possibly, due to partial availability of L-carnitine active substance not fully protected in the form used.

## 3. Biochemical blood indicators and non-specific immunity in Black Pied Holstein cows receiving dietary Carnipass™ ( $M \pm SEM$ , Dubrovitsy Farm, Experimental Farm Klenovo-Chegodaevo, 2014)

Indicator	Group			
	I (control) ( $n = 3$ )	II ( $n = 3$ )	III ( $n = 3$ )	IV ( $n = 3$ )
Total protein, g/l	82.70 $\pm$ 5.86	83.80 $\pm$ 4.92	86.10 $\pm$ 6.17	85.90 $\pm$ 5.98
Albumins, g/l	28.60 $\pm$ 1.47	30.70 $\pm$ 1.54	32.80 $\pm$ 1.76	32.60 $\pm$ 1.81
Globulins, g/l	54.10 $\pm$ 3.24	53.10 $\pm$ 4.23	53.30 $\pm$ 4.89	53.30 $\pm$ 5.14
Albumin/globulin ratio	0.53 $\pm$ 0.08	0.57 $\pm$ 0.10	0.62 $\pm$ 0.09*	0.61 $\pm$ 0.11*
Urea, $\mu$ mol/l	4.80 $\pm$ 0.98	3.70 $\pm$ 0.65	3.60 $\pm$ 0.72	3.60 $\pm$ 0.76
ALT, IU/l	20.10 $\pm$ 3.16	21.70 $\pm$ 2.87	22.40 $\pm$ 3.47	22.20 $\pm$ 3.54
AST, IU/l	85.10 $\pm$ 5.43	89.30 $\pm$ 6.07	91.60 $\pm$ 6.75	91.30 $\pm$ 6.72
Glucose, $\mu$ mol/l	3.20 $\pm$ 0.23	3.30 $\pm$ 0.18	3.50 $\pm$ 0.12	3.40 $\pm$ 0.15
Bilirubin, $\mu$ mol/l	4.70 $\pm$ 0.19	4.60 $\pm$ 0.15	4.50 $\pm$ 0.12	4.50 $\pm$ 0.09
Alkaline phosphatase, IU/l	98.40 $\pm$ 18.34	96.50 $\pm$ 16.67	83.70 $\pm$ 17.92	84.01 $\pm$ 19.17
Cholesterol, $\mu$ mol/l	4.80 $\pm$ 0.21	4.60 $\pm$ 0.32	4.30 $\pm$ 0.16	4.40 $\pm$ 0.18
Calcium, $\mu$ mol/l	2.40 $\pm$ 0.13	2.50 $\pm$ 0.28	2.80 $\pm$ 0.31	2.70 $\pm$ 0.26
Phosphorus, $\mu$ mol/l	1.50 $\pm$ 0.15	1.50 $\pm$ 0.19	1.60 $\pm$ 0.18	1.60 $\pm$ 0.18
Bacterial activity, %	78.40 $\pm$ 1.12	79.10 $\pm$ 0.11	85.30 $\pm$ 0.64***	85.20 $\pm$ 0.78***
Lysozyme activity, %	35.20 $\pm$ 2.34	35.90 $\pm$ 2.65**	40.60 $\pm$ 2.86**	40.50 $\pm$ 2.92**
Phagocyte index	3.70 $\pm$ 0.12	3.90 $\pm$ 0.16**	4.60 $\pm$ 0.11**	4.50 $\pm$ 0.15**
Phagocyte number	2.90 $\pm$ 0.11	3.20 $\pm$ 0.19	3.50 $\pm$ 0.17	3.50 $\pm$ 0.13
Phagocyte activity, %	66.20 $\pm$ 4.24	66.90 $\pm$ 4.87	68.30 $\pm$ 4.52	68.40 $\pm$ 4.63

Note. See description of groups in *Techniques* section. A/G — albumin to globulin ratio, ALT — alanine aminotransferase, AST — aspartate aminotransferase,

\*, \*\*, \*\*\* Differences from control are statistically significant at  $p < 0.05$ ,  $p < 0.01$  и  $p < 0.001$ , respectively.

Better digestibility of feed nutrients under the influence of carnitine promoted better metabolism of body substances and rendered positive effect on protein, carbohydrate and fat and mineral metabolism (Table 3). Improvement of nitrogen metabolism in animals supplied with L-carnitine was evidenced by growth of the total blood protein (by 1.5-4.6 % compared to control) and pro-

tein index (by 9.4-17.0 %), in addition to increased activity of transamination ferments (by 8.1-11.5 % for ALT and by 4.9-7.6 % for AST). Urea concentration decreased by 30.1-35.5 %, the least value noted in blood of cows from group III with 45 mg of L-carnitine/kg milk.

Increase in glucose by 4.7; 9.8 and 8.2 % as compared to control in blood of animals from groups II, III, and IV could evidence on improvement of the energy supply of cows getting carnitine. Alkaline phosphatase concentration decreased by 2.5; 8.6, and 8.3 %. Total blood bilirubin was 1.4; 4.7, and 4.9 % lower, cholesterol was 3.9; 8.8 and 8.2 % lower, which may evidence of acceleration of lipid metabolism and liver functions. Non-specific immunity values (phagocyte, bacterial, and lysozyme activity) increased by 2.08; 6.91, and 5.43 % in cows fed with carnitine (45 mg/kg of milk).

Comparison of biochemical and immunological indicators, evidencing on improvement of metabolic processes, shows the dietary protected L-carnitine in a dosage of 45 mg/kg of produced milk to be optimal. Farm test has shown that administration of L-carnitine in the said dosage had promoted increase of the average daily milk yield in absolute value and as per 4 %-fat milk by 10.6 and 11.6 %, respectively, compared to the control ( $p < 0.05$ ) (Table 4). High milk yield with the use of the said dosage of protected L-carnitine are coherent with results obtained in out experiment. At that, we did not reveal significant differences in content of fat, protein, lactose in milk of the experimental animals.

#### 4. Productive performance and milk composition in Black Pied Holstein cows receiving dietary Carnipass™ in farm testing ( $M \pm SEM$ , Agrofirma Detchinskoye LLC, Maloyaroslavetsky District, Kaluga Region, 2015-2016)

Indicator	Group	
	I (control) ( $n = 25$ )	II ( $n = 25$ )
Average daily milk yield, kg	30.30±0.58	33.50±0.69*
Average daily milk yield, % to control	100.0	110.6
Fat content in milk, %	4.08±0.19	4.12±0.21
Fat content in milk, % to control	100.0	101.0
Average daily yield as per 4 %-fat milk, kg	30.90±0.53	34.50±0.65*
Average daily yield as per 4 %-fat milk, % to control	100.0	111.6
Total yield of 4 %-fat milk, kg	3708	4140
Total yield of 4 %-fat milk, % to control	100.0	111.6
Protein, %	3.24±0.23	3.30±0.31
Protein, % to control	100.0	101.9
Lactose, %	4.74±0.02	4.76±0.02
Lactose, % to control	100.0	100.4

Note. See description of groups in *Techniques* section.

\* Differences from control are statistically significant at  $p < 0.05$ .

Used energetic feed units, digested protein and concentrates per unit of production in animals getting carnitine were 12.2, 11.8, and 11.4 % lower than in control.

During the 1<sup>st</sup> month after calving, control and experimental groups showed maximum loss of body weight (16.9 and 11.6 kg) (Table 5). During the 2<sup>nd</sup> months of lactation, loss of body weight in cows receiving carnitine decreased to the least extent than in the control ( $p < 0.05$ ). During days in milk, a 29.9 kg decrease from the initial weight was noted in control group, whereas it was lower in the experimental group (19.9 kg, difference of 2.9 %,  $p < 0.05$ ). Average daily live weight gain of 63 g in the experimental group as compared to control occurred on month 3 of lactation, which generally evidences of improved use of feed nutrients and energy when dietary L-carnitine was fed.

When protected L-carnitine (45 mg/kg of milk) was added to the diet, number of pregnant cows increased by 16 % for two sexual cycles, which was also reflected in better insemination index which decreased by 0.5 units and short-

er service period (by 24 days). During farm testing, surplus from milk sale was 3808 rubles per cow upon use of Carnipass (dosage of L-carnitine was 45 mg of active substance per 1 kg of produced milk).

**5. Body weight and its change after calving in Black Pied Holstein cows receiving dietary Carnipass™ in farm testing ( $M \pm SEM$ , Agrofirma Detchinskoye LLC, Maloyaroslavetsky District, Kaluga Region, years 2015-2016)**

Показатель	Group	
	I (control) (n = 25)	II (n = 25)
Body weight, kg:		
before launch	596.0±24.7	601.0±24.8
before calving	676.0±25.9	683.0±26.1
after calving		
in 5 days	575.0±22.6	581.0±23.9
in 1 month	558.1±22.8	569.4±23.1
in 2 months	546.4±22.5	561.1±22.6
in 3 months	545.1±21.9	563.0±23.5
in 4 months	548.5±22.5	574.2±23.4
Change of the body weigh after calving, kg:		
for 1 month	-16.9±1.5	-11.6±1.2
for 2 months	-11.7±1.3	-8.3±1.2
for 3 months	-1.3±0.7	+1.9±0.8
for 4 months	+3.4±0.8	+11.2±1.1

Note. See description of groups in *Techniques* section.

Our findings are in line with other data. Thus, the same milk yields were obtained in Red Pied Holstein cows upon use of dietary Carnipass™ (15 g · animal<sup>-1</sup> · day<sup>-1</sup>, CJSC Rus, Timashevsky District, Krasnodar Territory) [45, 46]. D.W. LaCount et al. [31] had established that upon addition of carnitine (nearly 6 g · animal<sup>-1</sup> · day<sup>-1</sup>) to the diet with 3 % raw fat, its blood concentration and content in liver tissues in cows had increased with improved lipid digestibility. V.N. Romanov et al. [47] report on positive physiological and productive effects of carnitine on calves (15 g · animal<sup>-1</sup> · day<sup>-1</sup>): improved metabolic processes in fore-stomach, increased digestibility of nutrients and retention of nitrogen in general contributed to more intensive growth in animals. Yu.P. Fomichev et al. [48] report that complex use of biologically active substances, the L-carnitine (Carnipass™), choline chloride and dehydroquercitine (Ecostimul-2), during interlactation period and at beginning of lactation not only enabled to prevent ketosis in highly productive cows, but also rendered positive effect on their milk yield and survivability. It is known that carnitine plays an important role in energy metabolism. Upon studying of the effect of additionally fed carnitine it was shown that it is accessible for ruminants even in non-protected form. Carnitine also ensures protection against toxic action of ammonium, which is observed at consumption of non-protein nitrogen or feed rich in soluble nitrogen [6].

Thus, use of protected carnitine in diets of highly productive cows during the most important periods of their physiological cycle, the interlactation phase and new-calving, promotes intensification of metabolism by improvement of microbial processes in fore-stomach and an increase in feed digestibility. Carnitine intensifies protective body functions, improves nitrogen metabolism, renders positive effect on protein, carbohydrate and fat, as well as mineral metabolism, and promotes growth of milk yield and reproductive values. The dosage of protected dietary L-carnitine of 45 mg of active substance per 1 kg of produced milk we deemed optimal for newly-calved cows.

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