DYNAMICS OF BLOOD PLASMA OSTEOTROPHIC ELEMENTS IN PULLETS AND LAYING HENS DURING CONTINUAL APPLICATION OF PROBIOTICS LACTIFERM

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Experimental application of probiotics Lactiferm was analysed in laying hybrids Shaver Starcross 288. There were control and three experimental groups. In control and experimental pullets blood was collected three times (39th, 101st and 133rd day), in laying hens four times (16th, 26th, 33rd and 49th laying week). In heparinized blood plasma the calcium and inorganic phosphorus concentration was estimated. Calcium concentrations in experimental pullets and laying hens changed insignificantly ($P < 0.05$). Inorganic phosphorus concentrations increased in experimental hens, especially in laying hens E2 supplemented by probiotics Lactiferm only during the rearing phase.

Gallus domesticus; pullets; laying hens; nutrition; probiotics; Lactiferm; blood; plasma; calcium; inorganic phosphorus

INTRODUCTION

Quoting more than six sources, Wolford and Tanaka (1970) reported that egg shell quality of chickens whether measured by specific gravidity, shell thickness, shell smoothness, breaking strength, percentage of cracks or shell appearance has been reported to be influenced by dietary calcium. High production in hen is related to calcium protein and energy intake (Davidson, Boyne, 1970; Soares, 1984; Nys et al., 1986; Proudfoot, Hullan, 1987; Härtl, 1990; Sazzad, Bertechini, 1994; Řezáč et al., 1997). The effects of calcium on the improvement of egg parameters and the deleterious effects induced by dietary calcium deficiency were studied and investigated by so many authors. A deficiency of calcium in diet brings about a progressive thinning of the shell followed by a complete cessation of laying, probably as a result of an inhibition of pituitary gonadotropin secretion. Reproduction in birds is accomplished by the production of a heavily calcified cleodoic egg (Hurwitz, Bornstein, 1966). Modern strains of domestic fowl produce up to 300 eggs per year and in the first months of production lay one egg a day for long periods.

This considerable output of calcium requires complex mechanisms so that sufficient calcium is obtained from the diet to maintain a correct calcium balance (Dewar et al., 1984).

It has been demonstrated that hens have a specific, diurnal appetite for Ca resulting from the ovulatory. The Ca appetite, peaking within several hours of dusk, precedes the need for Ca during the period of shell calcification and occurs even when shell formation is prevented by ligating the oviduct. Thus, the Ca requirement appears to be a major factor controlling food consumption on egg-forming days (Taylor, 1972). Leeson et al. (1978) reported greater consumption of Ca by hens on days when the oviposition occurs. This report is in line with previous findings by Hughes (1972) and Mongin, Mueller (1974).

It is well known that the amount of calcium and phosphorus required by laying hens depend on their stage of production and, therefore, vary not only from day-to-day but also throughout the day (Mongin, Mueller, 1974; Härtel, 1987).

In most of the experiments reported, the calcium supplement, usually limestone, was added to the expense of dietary grains. Thus, with the addition of calcium, the dietary energy level was lowered. Furthermore, limestone also changes the physical appearance of the diet and its specific gravity, which may influence feed intake by chickens (Hurwitz, Bar, 1971).

Influences of probiotics analysed marginal to dietary interactions of osteotrophical elements (calcium and inorganic phosphorus) only Mohan et al. (1966).

MATERIAL AND METHODS

Experiments took place at the Common Farm for Eggs Production (SZP) Markovice (Agrokomplex Kutná Hora). Precise specifications about experimental animals, housing, welfare, nutrition, research goals (Koudela, Nyirenda, 1995).

During each collection, from 8.00 h to 10.45 h, 6 pullets or layers from each group were killed. For pullets, the following groups are concerned: C1, C2, E1 and E3 and for layers, groups C1, E1, E2 and E3, were involved. In all 72 pullets and layers were killed. Blood was collected by cardial punction and death occurred through loss of blood. As an anticoagulant heparin in

solution was used (HEPARIN SPOFA – HEPARINUM NATRICUM – 50 000 IU in 10 ml of solution). From completely noncoagulated blood samples for haematological analysis were taken, heparinized blood plasma was collected after centrifugation.

Concentrations of calcium and inorganic phosphorus, according to methods of Homolka (1971) were used in the trial.

Obtained experimental results were evaluated according to regular variational method of statistics and the basic characteristics of mathematical selective sampling were determined.

RESULTS AND DISCUSSION

Resorption, deposition and mobilization, especially of calcium and phosphorus, have in layer hybrid maximal theoretical and practical meaning. In layer, during egg shell formation, remarkable relations take place between the supply of exogenous calcium and phosphorus in feed mixture, at one hand, and endogenous mobilization of bone and deposition in the egg shell on the other (Hertelendy, Taylor, 1961; Hurwitz, 1965; Hurwitz, Bar, 1966).

Dynamics of plasma calcium concentration

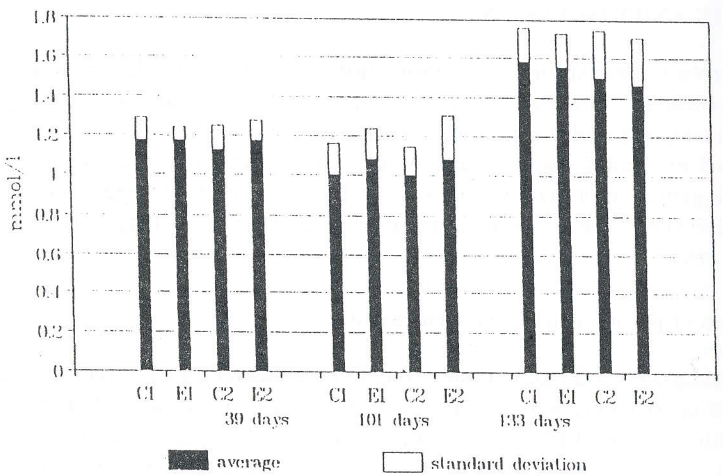
For adequate shell strength and objective avoidance of eggs with damaged or spoilt shell (shell with cracks), it is important to ensure a homeostasis, preferably calcium. From the average amount of 2.32 g of calcium accepted per bird and day, 1.81 g (that is to say 78%) is, during laying, resorbed and besides laying 1.76, that is to say 76% (Mueller et al., 1964). The degree of layer pullets and layer organism saturation with calcium is therefore analyzed through determination of calcium concentrations in heparinized blood plasma. Tab. I summarizes results obtained from plasma calcium concentrations in control and experimental layer pullets.

Obtained results on calcium concentrations did not significantly differ in both experimental and both control groups of layer pullets (Fig. 1).

Plasma calcium, at the upper limit of concentrations, reached in control pullets basic physiological values (Sturkie, Mueller, 1976; Krampitz et al., 1983). Changes in blood plasma calcium concentrations are more different for layers than pullets. In average, the egg shell contains, in the same way, 1.7–2.1 g Ca. During 85% of laying level, and during an average amount of 2 g Ca in egg shell, layers secrete off, every ten days, the entire endogenic calcium amount (17 g) in form of shell calcium. In the main phase of egg shell mineralization, 100 mg (25 mmol) of calcium is, every hour, eliminated

I. Dynamics of blood plasma calcium concentrations in pullets ($mmol.l^{-1}$)

Age (days)	Group											
	C1			E1			C2			E2		
	$\bar{x}$	s	v%	$\bar{x}$	s	v%	$\bar{x}$	s	v%	$\bar{x}$	s	v%
39	2.33	0.32	14	2.03	0.41	20	2.15	0.33	15	2.16	0.25	12
101	1.96	0.19	10	1.95	0.28	15	1.93	0.26	14	1.91	0.26	14
133	2.86	0.37	13	2.05	0.30	15	2.23	0.50	23	2.58	0.64	19



1. Blood plasma calcium concentrations in pullets

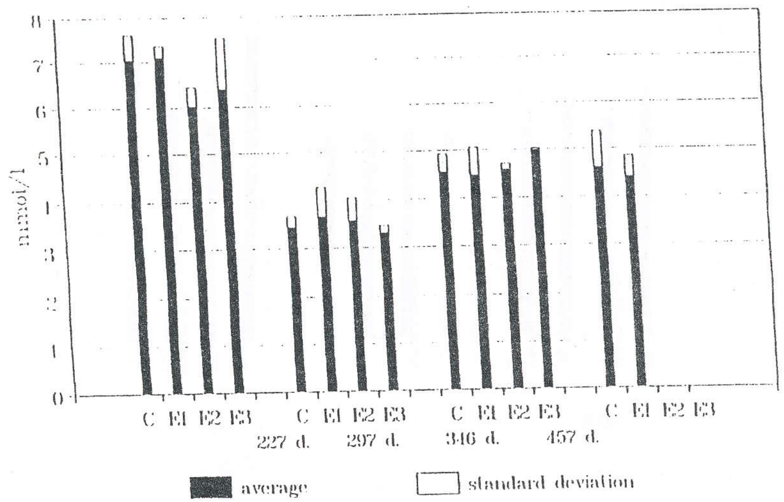
from blood plasma and the entire quantity is remarkable reduced by 1–1.25 $mmol.l^{-1}$ (Hertelendy, Taylor, 1961). During laying period, blood plasma calcium concentrations in control and experimental layers changed (Tab. II).

Plasma calcium concentrations changed during laying phase (Fig. 2) however remained different for individual groups of layers. In control layers, the reduction in calcium concentrations (about 35%) took place at the age between 227 and 346 days. In all three experimental groups of layers, this decline was 27%.

During the period under studies, the reduction in calcium concentrations in experimental layers, which received in feed mixtures, Lactiferm through

II. Dynamics of blood plasma calcium concentrations in layers ($mmol.l^{-1}$)

Age (days)	Group											
	C1			E1			C2			E2		
	$\bar{x}$	s	v%	$\bar{x}$	s	v%	$\bar{x}$	s	v%	$\bar{x}$	s	v%
227	7.04	0.60	9	7.10	0.28	4	6.01	0.43	7	6.38	1.16	18
297	3.43	0.25	7	3.64	0.64	18	3.56	0.50	14	3.29	0.15	5
346	4.55	0.38	8	4.48	0.57	13	4.59	0.14	3	4.98	0.04	1
457	4.61	0.74	16	4.41	0.45	10	layers sent to slaughterhouse					



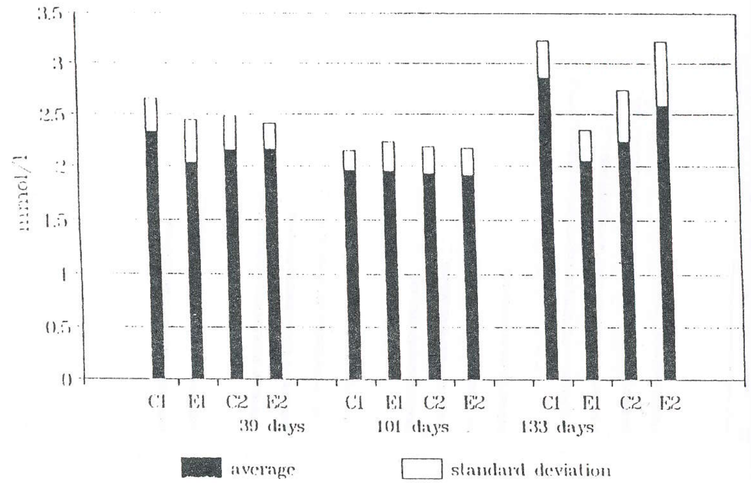
2. Blood plasma calcium concentrations in laying hens

the whole period of experiment, that is to say, during pullets' rearing and eggs laying period, stood at 37%, in experimental group E2 (probiotics applied during pullets rearing only), decline in calcium concentrations was about 24%. In experimental group E3, with probiotics Lactiferm applied during eggs laying period only, reached the decline in plasma calcium concentrations, through 120 days under studies, a reached mere 22%.

Consequently, changes in calcium concentrations manifested themselves only in those experimental layers fed on feed mixtures supplemented with Lactiferm during pullets rearing or eggs laying period. Similarly, in control and experimental pullets, calcium concentrations, in layer, were not different

III. Dynamics of blood plasma inorganic phosphorus concentrations in layer pullets (mmol.l^{-1})

Age (days)	Group											
	C1			E1			C2			E2		
	$\bar{x}$	s	v%	$\bar{x}$	s	v%	$\bar{x}$	s	v%	$\bar{x}$	s	v%
39	1.17	0.12	11	1.17	0.07	6	1.12	0.13	12	1.17	0.11	9
101	1.00	0.16	16	1.08	0.16	15	1.00	0.14	14	1.08	0.23	22
133	1.58	0.18	11	1.55	0.18	11	1.50	0.25	17	1.46	0.25	17



3. Blood plasma phosphorus concentrations in pullets

from basic physiological values (S t u r k i e, M u e l l e r, 1976; K r a m p i t z et al., 1983).

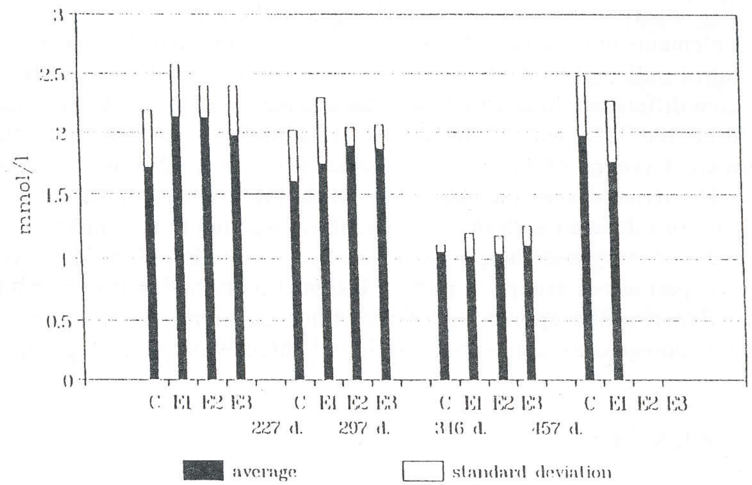
Dynamics of blood plasma phosphorus concentrations

Polyfactorial physiological characteristics of phosphorus and low relations calcium – phosphorus (H u r w i t z, 1964) were the cause behind concentrated interest in the interpretation of changes in phosphorus concentrations in heparinized blood plasma. Tab. III summarizes the results of phosphorus (P) concentrations determination in blood plasma.

During pullets rearing, phosphorus concentrations slightly increased (Fig. 3). Just like in case of blood calcium concentrations, a slight decline in

IV. Dynamics of blood plasma inorganic phosphorus concentrations in layer (mmol.l^{-1})

Age (days)	Group											
	C1			E1			C2			E2		
	$\bar{x}$	s	v%	$\bar{x}$	s	v%	$\bar{x}$	s	v%	$\bar{x}$	s	v%
227	1.70	0.48	28	2.13	0.45	21	2.12	0.27	13	1.97	0.42	21
297	1.59	0.42	26	1.73	0.56	32	1.88	0.16	9	1.85	0.21	12
346	1.05	0.06	6	1.02	0.18	17	1.04	0.14	13	1.10	0.16	14
457	1.96	0.52	27	1.74	0.52	29	layers sent to slaughterhouse					



4. Blood plasma phosphorus concentrations in laying hens

phosphorus concentrations was in control and experimental pullets at the age of 101 days, reported. The cause of this decline could be probably found in the metabolism break down following the outbreak of the earlier disease. Interestingly, the blood plasma phosphorus concentrations remained insignificantly higher in experimental groups when compared to the control pullets.

During laying period, blood plasma phosphorus concentrations (Fig. 4) declined in all experimental and control groups between 227 and 346 days of age. Blood collection at the end of the experimental was from the control pullets and one experimental group (E1) only. Observed rise in phosphorus concentrations, which showed great biological variabilities, is here very difficult to interpret.

Tab. IV gives a view on blood plasma phosphorus concentrations in layers. Obtained results can be, generally, considered as basic (Krampitz et al., 1983).

During evaluation of probiotics Lactiferm, at the level of phosphorus metabolism, a slight increase in the blood plasma phosphorus concentration (Fig. 4) was observed in all three experimental groups of layers. Spread out time intervals for the application of Lactiferm did not show statistically any significance in the rise of phosphorus concentrations.

CONCLUSIONS

During experimental period, some changes in the concentrations of osteotrophic elements in heparinized blood plasma were observed. In experimental and control pullets, blood plasma calcium concentrations did not show any significant difference. In control layers, these concentrations declined remarkably during the 227th and 346th day of layer's life by about 35%. In all three experimental groups of layers, this decline was lower (27%) than control layers. The dynamics in the decline of heparinized blood plasma calcium concentrations differed with the time Lactiferm was fed to the animals. Concentrations of inorganic phosphorus in the heparinized blood plasma was, in the two experimental groups of pullets, insignificantly higher than in control pullets. Experimental application of probiotics Lactiferm slightly increased inorganic phosphorus concentrations in all three experimental groups of layers.

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Dynamika osteotrofických prvků v krevní plazmě kuřic a nosnic při experimentální aplikaci probiotika Lactiferm.

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Probiotikum Lactiferm bylo kontinuálně aplikováno kuřicím a nosnicím snáškového hybrida Shaver Starcross 288. Vedle kontrolních skupin se vytvořily tři skupiny pokusné. Pokusná skupina E1 dostávala probiotikum Lactiferm po celou dobu pokusu, tzn. jak v období odchovu kuřic, tak v etapě snášky, pokusná skupina E2 dostávala probiotikum pouze v období odchovu. Pokusnou skupinu E3 tvořily nosnice, které dostávaly probiotikum pouze v období snášky.

Kontrolním a pokusným kuřicím byla odebrána krev ve věku 39, 101 a 133 dnů, nosnicím byla odebrána krev v 16., 26., 33. a 49. týdnu snášky. Krev byla odebírána v době od 8.00 do 10.45 hodin, jako protisrážlivý prostředek byl použit heparin v substanci (Heparin Spofa – Heparinum natricum). V heparinizované krevní plazmě byly kolorimetricky stanoveny koncentrace vápníku a anorganického fosforu (H o m o l k a , 1971).

Plazmatické koncentrace vápníku i fosforu kontrolních kuřic i nosnic se pohybovaly v rozsahu základních fyziologických hodnot. U pokusných a kontrolních kuřic se koncentrace obou osteotrofických prvků výrazněji neměnily. Aplikací probiotika Lactiferm se ve sledovaném období koncentrace vápníku v krevní plazmě nosnic statisticky významně nezměnily. Hodnoty koncentrace anorganického fosforu v krevní plazmě pokusných nosnic se ve srovnání s kontrolními nosnicemi zvýšily, a to nejvýrazněji u nosnic E2, které probiotikum dostávaly pouze v období odchovu.

Gallus domesticus; kuřice; nosnice; výživa; probiotika; Lactiferm; krev; plazma; vápník; anorganický fosfor

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