INFLUENCES OF PROBIOTICS LACTIFERM ON METHAEMOGLOBIN BIOSYNTHESIS IN *GALLUS DOMESTICUS*

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During the continual experimental application of probiotic Lactiferm a rapid decline in methaemoglobin biosynthesis in the erythrocytes of all experimental groups was found in pullets and laying hens Shaver Starcross. The experiments took place in an area known for chronic concentrations of nitrates in drinking water (74 to 165 mg/l). Methaemoglobin concentrations in the erythrocytes of laying hens were on an average about 26% lower than the control ones.

Gallus domesticus; pullets; laying hens; nutrition; probiotics; Lactiferm; blood; erythrocytes; haemoglobin; methaemoglobin

INTRODUCTION

Optimization of nitrogenous feed for agricultural plants is, at the present time in the Czech Republic, highly topical. Misuse of the industrial N-fertilizers modifies the natural movement of nitrogen. Misuse and irresponsible increase in the use of nitrogenous fertilizers can cause risky situations, which are connected with the increase of nitrate and nitrite levels in both surface and underground water (Petr, Dlouhý, 1992). Agricultural produces use, according to circumstances 10–90% of applied N-fertilizers. Citing the work of Stibral (1986), Koudela et al. (1990) reported that in ČSFR 25% recorded the peak of harvest which reached on the level of 220% contamination of ground water by nitrates. Hence, it formed a nitrate load on the agrosystem, which could be analyzed in free living and domestic animals.

Nitrites are by far dangerous than nitrates and are produced by intestinal microbial reduction. The danger of nitrites in vertebrates consists, apart from other reasons, in haemoglobin oxidation to methaemoglobin (MHb). MHb-biosynthesis results from the transformation of the double bonded iron (ferrous) into triple bonded one (ferric form), which is not capable of riversibly binding and releasing oxygen. MHb was described by Gamgee (cit. Koudela, 1991), some 125 years ago (1868). The complete reaction for MHb

formation, which has not been up to now fully elucidated, was expressed by Yano et al. (1982) as follows:

$$4\ HbO_2 + 4\ NO_2 + 4\ H^+ -> Hb^3 + 4\ NO_3 + O_2 + 2\ H_2O$$

In erythrocytes a small quantity of MHb is continuously formed, which is gradually reduced to haemoglobin. The human blood usually contains the total haemoglobin concentrations, not exceeding 0.1 to 1% MHb. An increase in MHb concentration is termed methaemoglobinaemia. At the present, it has been studied and the existence of MHb was causally found in humans, particularly in suckling human infants, and domestic animals, for instance horned cattle. In other animal species, information on MHb dynamic concentrations is very scarce, be it in domestic or free living animals.

For the past 15 years, an extensive study of the dynamics of methaemoglobinaemia in domestic fowl, turkey-hen, dogs, pigs, common carp and from free living animals, hare, wild rabbit and pheasant, was carried out. In some conditions, it was found as a useful common indicator, the field hare, which represents a territorial faithfulness, because of its connection to agrobiocenosis in an exclusive area of 300 m^2 (Koudela, 1991).

Stimulative results obtained through monitoring the nitrates load on agroecosystem by using free living animals, led us, in 1980 (Koudela, 1991), to include MHb to the paletot of blood indicatory constituents during analysis of metabolic responses in domestic fowl when screening non-traditional sources of proteins and energy for poultry feed.

There is no knowledge about interactions HMb biosynthesis in pullets and laying hens during the experimental continual feeding of lactacidogenic microbes. The research has been aimed at explaining possible influences of probiotics Lactiferm.

MATERIAL AND METHODS

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

It is very interesting, that water sources near the town Čáslav, the Kutná Hora district, are marked by high concentrations of nitrates and nitrites, whose source is the dung from piggeries from nearby state farm within the same area of Čáslav. Up to 1983, the nitrates concentrations in drinking water of Markovice ranged around 50 mg/l and rose three times higher (100–150 mg/l) at the time when our experiment began.

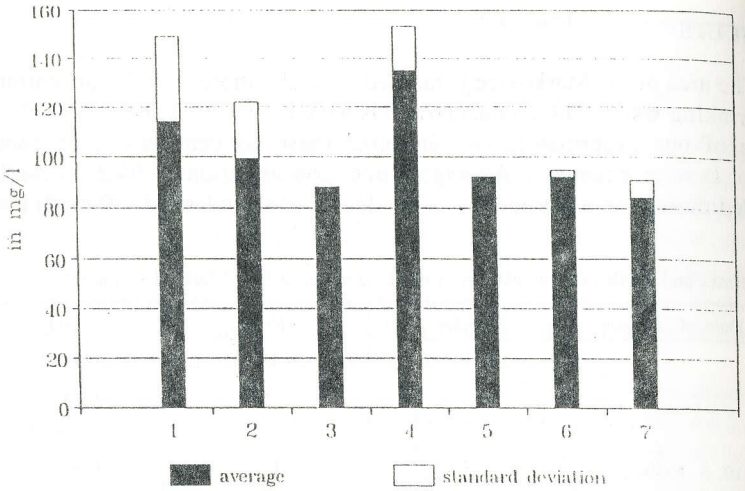
The concentrations of nitrates, nitrites, haemoglobin and methaemoglobin in the control and experimental pullets and laying hens were determined according to exact methods (Homolka, 1971).

RESULTS

The area of the Markovice is marked by high nitrates (NO_3^-) concentrations in drinking water. These concentrations were between 74 and 165 mg/l at the time of our experiment. To determine these concentrations, 21 samples (Tab. I) were examined. Average nitrate concentrations (104 ± 27 mg/l) recorded during our experiment was the double of the tolerated (50 mg/l) level.

I. Nitrates and nitrites concentrations in drinking water at SZP Markovice (mg/l)

Date of analysis	Samples	NO_3^-	NO_2^-
19. 6. 1989	1	165	traces
	2	97	traces
	3	76	not found
	4	152	traces
	5	120	traces
	6	114	not found
	7	74	traces
	1–7	114 ± 35.15	
20. 9. 1989	8	83	
	9	115	
	8 + 9	99 ± 22.62	
20. 10. 1989	10	80	0.01
13. 11. 1989	11	124	not found
	12	149	traces
	13	116	traces
	1–13	135 ± 17.78	
24. 1. 1990	14	92	not found
3. 4. 1990	15	90	0.25
	16	94	not found
	17	92	not found
	15–17	92 ± 2.82	
25. 5. 1990	18	75	traces
	19	87	traces
	20	92	not found
	21	81	traces
	18–21	84 ± 7.36	



1. Dynamics of nitrates concentrations in drinking water

Nitrate concentrations did not fall below the maximal allowed concentrations of 50 mg/l (Fig. 1). It is worth mentioning that some traces of nitrites as well as some high levels of nitrate concentrations (0.25 ± 0.01 mg/l) were recorded (Fig. 2).

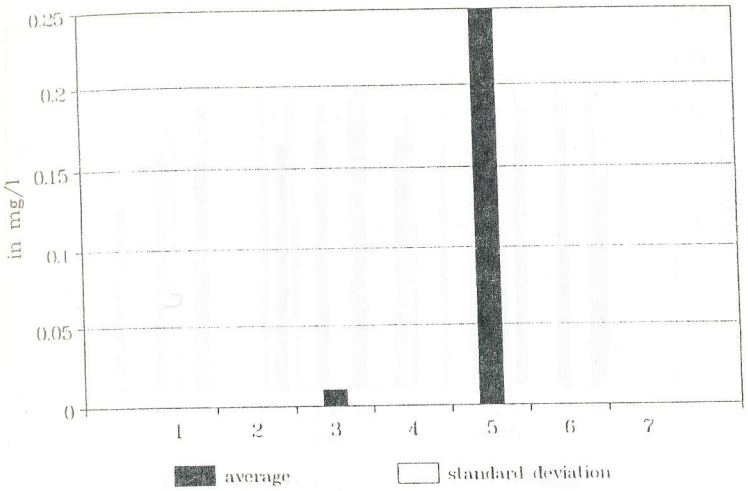
It is of no doubt that, increased level of nitrates as well as nitrite contaminations of drinking water, could not be the cause of haemoglobin (Hb) degradation to harmful MHb.

Haemoglobin concentrations, in layer pullets erythrocytes (Tab. II) ranged between 6.92 and 11.00 mmol/l and reached the basic physiological values (Sturkie, 1976; Mehner, Hartfiel, 1983).

Haemoglobin concentrations between experimental and control groups did not show any significant difference. The only statistical significance ($P < 0.05$) was recorded in E1 group at the age of 101 days. (Fig. 3).

II. Dynamics of haemoglobin concentrations in pullets (mmol/l)

Age in days	Group											
	C1			E1			C2			E2		
	$\bar{x}$	s	v%	$\bar{x}$	s	v%	$\bar{x}$	s	v%	$\bar{x}$	s	v%
39	7.53	1.45	19	9.43	1.62	17	10.28	1.99	19	9.82	1.22	12
101	9.27	9.72	8	11.00	1.04	9	9.27	0.63	7	9.00	1.42	16
133	8.80	1.36	15	8.88	1.56	18	6.92	0.22	3	7.48	0.62	8



2. Dynamics of nitrites concentrations in drinking water

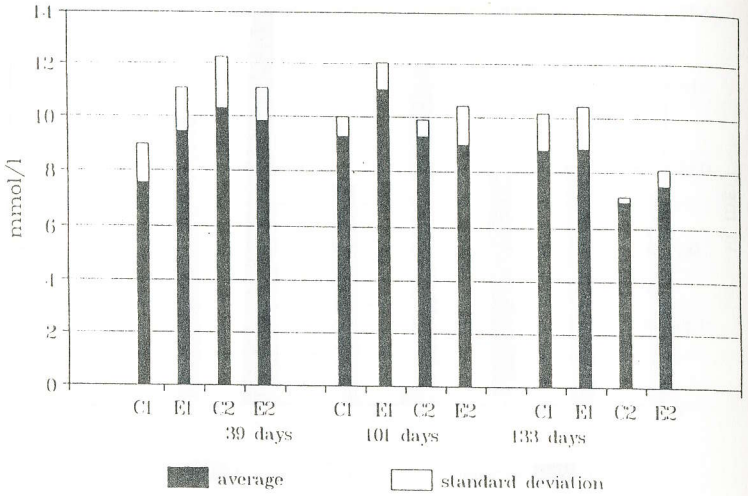
Long-lasting intake of drinking water with high nitrate concentration caused the occurrence of significant MHb concentrations in pullets (Fig. 4) of the experimental as well as control groups at 101 days of age. We also observed that almost between 1/4 to 1/5 of haemoglobin was oxidized to MHb (Tab. III, Fig. 5).

Haemoglobin concentrations in laying hens summarizes Tab. IV.

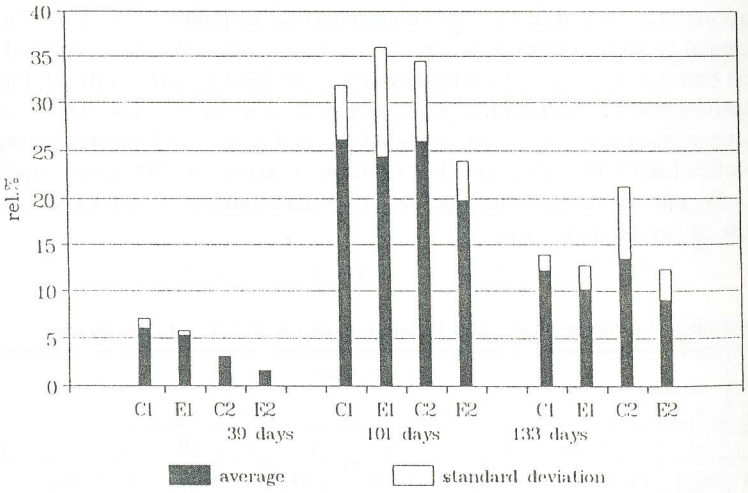
A very interesting situation notified here is that the continuous feeding on probiotics Lactiferm influenced both abrupt variations in MHb concentrations (Fig. 6) and its remarkable decline in concentrations in all experimental groups of laying hens (Tab. V).

III. Dynamics of methaemoglobin concentrations in pullets (rel. % in haemoglobin)

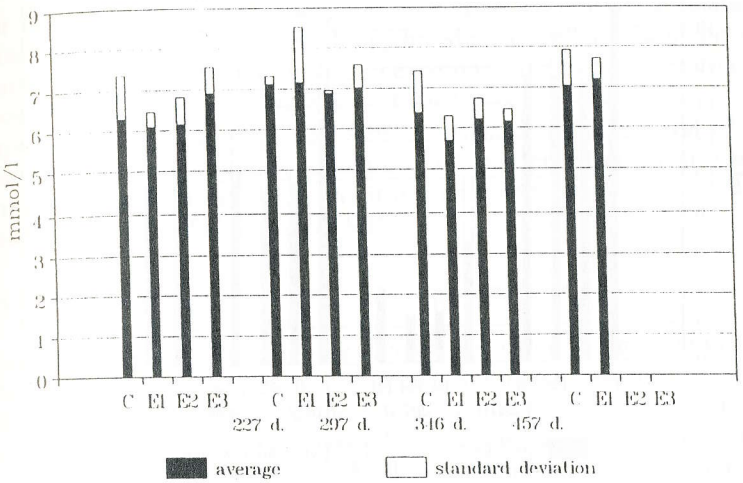
Age in days	Group											
	C1			E1			C2			E2		
	$\bar{x}$	s	v%	$\bar{x}$	s	v%	$\bar{x}$	s	v%	$\bar{x}$	s	v%
39	6.10	1.03	17	5.38	0.49	8	2.98	0.11	4	1.53	0.10	7
101	26.18	5.80	22	24.30	11.72	48	25.97	8.50	32	19.73	4.15	21
133	12.23	1.74	14	10.16	2.56	25	13.46	7.81	58	8.98	3.37	37



3. Dynamics of haemoglobin concentrations in pullets



4. Dynamics of methaemoglobin concentrations in pullets



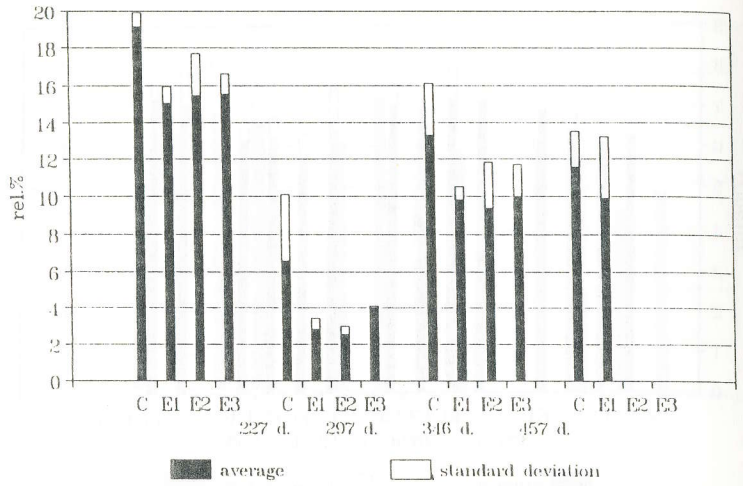
5. Dynamics of haemoglobin concentrations in laying hens

IV. Dynamics of haemoglobin concentrations in laying hens (mmol/l)

Age in days	Group											
	C1			E1			C2			E2		
	$\bar{x}$	s	v%	$\bar{x}$	s	v%	$\bar{x}$	s	v%	$\bar{x}$	s	v%
227	6.27	1.10	17	6.07	0.38	6	6.13	0.68	1	6.10	0.6	9
297	7.12	0.21	3	7.12	1.37	19	6.87	0.09	1	7.01	0.5	8
346	6.38	1.05	16	5.69	0.62	11	6.21	0.52	8	6.16	0.3	5
457	7.01	0.89	13	7.21	0.52	7	layers sent to slaughter house					

V. Dynamics of methaemoglobin concentrations in laying hens (rel. %)

Age in days	Group											
	C1			E1			C2			E2		
	$\bar{x}$	s	v%	$\bar{x}$	s	v%	$\bar{x}$	s	v%	$\bar{x}$	s	v%
227	19.13	0.81	4	15.03	0.95	6	15.47	2.25	14	15.56	1.12	17
297	6.60	3.50	53	2.80	0.61	22	2.53	0.45	13	4.00	0.10	0.25
346	13.30	2.85	21	9.80	0.70	7	9.40	2.45	26	9.96	1.70	17
457	11.55	2.03	17	9.87	3.35	34	layers sent to slaughter house					



6. Dynamics of methaemoglobin concentrations in laying hens

DISCUSSION

MHb concentrations ranged, in control pullets, during the whole period under studies, between 1.8 and 38.5 relative percentage of the total haemoglobin quantity (C1 + C2 = 14.48 ± 9.77 rel. %). A lower level of MHb concentrations was recorded in experimental pullets that ranged between 1.3 and 34.8 rel. % (E1 + E2 = 11.68 ± 8,67 rel. %). In total, MHb concentrations in the two experimental groups were approximately 20% lower than in both control groups of pullets.

In laying hens, the results obtained on Hb concentrations did not show any deviations from the basic physiological values. Due to organizational reasons, we were able to collect, during the fourth blood collection, on 11. 9. 1990, blood samples from control group of laying hens and one experimental (E1) group only. The rest of laying hens had already been taken for slaughtering.

MHb concentrations were always, in control layers, higher in contrast with those in experimental layers. MHb concentrations in the erythrocytes of laying hens fed on Lactiferm during both breeding and rearing period, were, on average about 25.88% lower than in the control layers. Laying hens fed on Lactiferm supplemented feed mixtures during the rearing period only, showed an average of 27.75%. MHb concentrations in the erythrocytes of layers fed on Lactiferm in feed mixtures starting from 150th day of bird's life, were, on average, about 22.16% lower in contrast with control laying hens.

For the whole period of experiment, that is to say during a continual application of probiotics Lactiferm during breeding as well as rearing period, examined MHb concentrations decline in experimental groups, on an average of about 20% (C – 13.75 ± 7.92 X E – 10.89 ± 7.16 rel. %).

Experimental application of Lactiferm contributed to the removal of the dangerous and harmful hypoxic anaemia which was provoked by an intake of drinking water with extreme high nitrite concentrations and the resulting degradation of Hb to the inefficient MHb.

CONCLUSIONS

Experiments took place in an area known for chronic concentrations of nitrates in drinking water (74 to 165 mg/l). Sensitively reacted red blood corpuscle pigment, to the exogenic intake of nitrates, which degrades to methaemoglobin (MHb). It was found, during a continual application of probiotics Lactiferm, a rapid decline in MHb concentrations in all experimental groups of pullets and laying hens. MHb concentrations in the erythrocytes of laying hens fed on probiotics Lactiferm during both breeding and rearing period were on average about 25.88% lower than in the control laying hens. In the erythrocytes of laying hens fed on Lactiferm, only during the rearing period this difference was on average 27.75%. In the third experimental group, which received Lactiferm continually in feed mixtures through the laying period, from the so-called 150th day of life, average MHb concentrations were about 22.16% lower compared to the control layers.

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Vlivy probiotika Lactiferm na biosyntézu methemoglobinu u kuřic a nosnic.

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Experimentální kontinuální aplikace probiotika Lactiferm kuřicím a nosnicím se uskutečnila v prostředí chronicky zatíženém dusičnany. Jejich koncentrace v pitné vodě kolísaly v rozsahu 74 až 165 mg/l, tzn. dvakrát až třikrát převyšovaly maximálně tolerované množství dusičnanů (50 mg/l). Dusičnany přijaté s pitnou vodou jsou mikroflórou ve střevě degradovány na dusitany, které podmiňují tvorbu nebezpečného methemoglobinu (MHb). Vznik MHb vyvolává anemickou hypoxii a následný deficit molekulárního kyslíku. Kontinuální aplikace laktacidogenních zárodků obsažených v probiotiku Lactiferm se projevila zajímavou ochranou erytrocytů kuřic i nosnic proti oxidačním účinkům dusičnanů a dusitanů.

Koncentrace hemoglobinu v erytrocytech kuřic i nosnic dosáhly základních fyziologických hodnot. U obou pokusných skupin kuřic se snížily ve srovnání s kontrolními kuřicemi koncentrace MHb, přičemž u kuřic ve věku 101 dní byl tento pokles statisticky významný ($P < 0,05$). Také u nosnic se aplikací probiotika Lactifermu snížily u pokusných zvířat koncentrace MHb, a to v průměru o 20 %. Pozoruhodný je signifikantní pokles koncentrace MHb u všech pokusných nosnic ve věku 297 dní, a to přibližně na poloviční hodnoty ve srovnání s nosnicemi kontrolními.

Gallus domesticus; kuřice; nosnice; výživa; probiotika; Lactiferm; krev; erytrocyty; hemoglobin; methemoglobin

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