

OBSERVATION ON METHEMOGLOBINAEMIA IN DAIRY COWS DURING FOUR YEARS

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Abstract

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During four years of investigation (time periods A, B, C, D) methemoglobinaemia has been observed in venous blood of 2 812 dairy cows, out of which 1 687 were fed winter feed rations and 1 125 animals received summer feed rations (fresh grass). This disorder was noted in the 1st and 2nd lactation stage and in 8th—9.5th month of pregnancy. The average methemoglobin values (MHb) slightly exceeded the tolerance limit of 5 % hemoglobin. Neither in the entire populations nor in individual groups of cows a significant difference was observed between all summarized summer and winter seasons. The methemoglobin value during winter season in highly pregnant cows was observed to be significantly lower than that in lactating cows. The difference of feed rations had only a little influence upon the level of methemoglobin, the effect of feed composition and quality in individual years being, however, significant. The highest significance of methemoglobin concentration was observed in all cow groups in the summer season of the A period and in the following winter season of the B period. The highest concentration in both these periods was observed in cows in the 2nd lactation stage ($7.27 \pm 1.47\%$ and $6.15 \pm 1.84\%$ in winter and summer season, resp.). In the last two years (C and D) methemoglobin values decreased in all groups of cows, this being more pronounced in winter than in summer seasons.

The influence of subclinical chronic methemoglobinaemia on the health condition of dairy cows and on economic parameters of husbandry is discussed.

Cows, methemoglobin, summer and winter feeding, 1st and 2nd lactation stage, pregnancy.

The values of methemoglobin presented in literature as being normal for healthy cattle vary considerably. According to Bartík and Rosival (1971) this value ranges from 0.3 to 1.7 % of total hemoglobin whereas Spörri and Stünzi (1969) presented the value of 3—5 %. Penkov (1974 - quot. Lebeda et al. 1978) reported the values reaching up to 3.7 % of hemoglobin but in calves at most four days old he found no methemoglobin. The tendency of the «normal» or tolerable methemoglobin values to increase is associated with the intensification of agriculture and the consequence of which are the increased nitrate levels in soil, water and crop (see Garner 1958; Bartík and Rosival 1971 and others).

The results of our previous research (Lebeda 1971; Lebeda et al. 1978; Lebeda and Kovařík 1978) have shown that in conditions of summer and winter feeding the methemoglobin levels exceed, in majority of dairy cows, the upper limit of contemporary «normal» values (i. e. 5 % of the total hemoglobin) by 2—4 %. Sporadically, even higher values have been observed. When comparing the findings with the conditions of soil fertilizing in the South moravian country region by artificial fertilizers a hypothesis has been put forward that the diagnosed cases of methemoglobinaemia are due to the permanent overloading of dairy cows with methemoglobin producing substances, especially with nitrates which are reduced to nitrites. The most probable cause of the increased nitrate concentration in crop appears to be a constantly growing fertilization by nitrogen-containing fertilizers and the application of herbicides that increase the nitrate content in plants. The average incidence of hemoglobinuria, which is the manifestation of substance having an

adverse effect upon the erythrocyte metabolism, ranged from 8.34 to 28.88 %, reaching the maximum at 5–7 % of methemoglobin. Most of the sets of examined cows fell into this range.

Even in subclinical cases of methemoglobinaemia the functionally eliminated hemoglobin must be compensated for by a more rapid blood circulation to the expense of reserve or productive energy (Lebeda et al. 1978). The elevated methemoglobin levels impair also the liver function, decrease the urea synthesis and increase blood ammonia and glycaemia (Myazaki and Kawashima 1975 - quot. Lebeda et al. 1978). The background of the increased methemoglobinaemia forms a dangerous situation in the case of further intake of methemoglobin-producing substances and of other metabolic disturbances. The combination of these adverse effects is negatively reflected in the milk production, body mass of animals, reproduction, abortions and total economics in nutrient utilization and in metabolic disturbances. A general picture of disturbances is thus formed, that cannot be simply classified into one of classical nosologic diseases (Lebeda et al. 1978). We have thus recommended to pay greater attention to the nitrate and nitrite levels in feeds and drinking water in all cases of the mentioned health disturbances in dairy cows with unclear etiology and of those exhibiting elevated levels of methemoglobin. At the same time it is necessary to reduce preventively feeding of plants with apparent signs of over-fertilization with nitrogen. During the time which elapsed since the publication of our results a considerable progress has been achieved as to the investigation of the nitrogen content in feedstuffs. At present the agrolaboratories provide an advisory service concerning a possible restriction in feed rations with a higher content of nitrates.

CSSR belongs to countries that are achieving a relatively high production of cereals per hectare, the cost however being paid by a high input of fertilizers. In the period of 1960–1970 the optimum amount of nitrogen-containing fertilizers was exceeded in some crop areas and the trend in yields has started to lag significantly behind the steeply increasing amount of nitrogen put into the average hectare of crop area. Thus, not only energy loss but also the disturbances of soil agrosystem appear (Stibral 1984). Ever increasing animal production requires higher amount of feeds, the providing of which is possible by intensification of crop production only. This leads to the rise in the fertilizer use even in the production of roughages.

In the period 1977–1982 the application of fertilizers has increased as follows: agricultural triad by 0.63 %, N by 10.86 %, P by 11.11 %, whereas in the case of K the application dropped by 0.83 % (Mareček 1984). The south-moravian country district, from which the majority of the examined dairy cows comes, reached roughly this level as early as in 1977. It can thus be assumed that the nitrate load on dairy cows was not lesser in the subsequent years.

Apart from the known nitrate and nitrite poisoning with the development of methemoglobinaemia (Lebeda et al. 1978) a serious risk exists in the reaction of nitrites with secondary and tertiary amines during which nitrosamines are formed, the latter exhibiting a clear cancerogenous effect upon a series of animals (Mareček 1984). The accumulation of nitrates in plants is dependent upon the weather conditions in the crop vegetation period and on the management system in individual agricultural enterprises. All factors, preventing the normal course of photosynthesis, hinder also the transformation of nitrates taken up by roots and the incorporation of N into the protein bond. In this way the nitrites are accumulated in the plants. The processes stimulating the photosynthesis induce, on the other hand, the decrease of nitrites in crops (Mareček 1984).

Nitrates themselves are relatively non-toxic, they cause rather the disturbance of osmotic equilibria in the organism, similarly as in the case of sodium chloride poisoning. They become toxic after the reduction to nitrites in feeds or in the gastrointestinal tract (Bartík and Rosival 1971). Nitrites are the intermediate product in the reduction of nitrates to ammonia in the rumen or in the intestine of other herbivores. When a certain nitrate concentration is exceeded, the speed of reduction to ammonia decreases and an accumulation of nitrites occurs. If the reduction of nitrates does not take place or if the reduction to ammonia is complete, the risk of intoxication is small (Bartík and Rosival 1971). Ruminants are more sensitive to nitrate poisoning since the rumen microflora facilitates the nitrate reduction. Any factor changing normal rumen microflora can facilitate the nitrate reduction and thus the intoxication (Fowler 1977). Non-transformed nitrates, nitrites and ammonia get rapidly from the rumen to blood. Here, the nitrites react with hemoglobin thus forming methemoglobin (Bartík and Rosival 1971). If the quality of feeds is good the cattle tolerate a higher amount of nitrates (up to 1.5 % in the dry matter) when compared with the foodstuffs that are poor and deficient (only 0.7 % - Case 1958). When the feed ration contains a high amount of carbohydrates, valuable protein and high level of nutrients the content of 2–5 % of nitrates is tolerated (Fowler 1977). Generally, the amount of nitrates per cow and day should not exceed 50 g and 0.5 % KNO_3 in the dry matter of a feed ration (Garner 1958). This amount does not induce any apparent changes in the health condition. The amount of 0.6–1.0 % KNO_3 decreases the milk production and after 6–8 weeks the signs of vitamin A deficiency appear. At the concentration of 1.0–1.5 % the milk production ceases after 4–5 days and reproduction disturbances, outlasting even several weeks after the removal of defective food-

stuffs, are observed. The concentrations exceeding 1.5 % KNO_3 in the dry matter of the feed ration cause usually a sudden death of several animals (Garner 1958).

Long lasting intoxication by sublethal or possibly subclinical doses of nitrates are regarded as dangerous as acute and lethal poisonings. Higher nitrate concentrations in feeds result in a lower milk yield, body mass loss in animals, noninfectious abortions and signs usually observed in vitamin A deficiency (Bartík and Rosival 1971).

The present study of the variability of methemoglobinaemia in cows in different lactation stage and reproduction cycle during four periods of fresh grass feeding and four periods of winter feeding has been motivated by economic and health consequences resulting from elevated nitrate levels in feeds under the conditions of intensive agricultural production. The aim was to determine the degree by which different conditions of crop vegetation and from it following feeds balance in individual years are manifested in the level of methemoglobinaemia.

Materials and Methods

During four years of metabolic examination the methemoglobin concentration in venous blood was determined in 2 812 cows, out of which 1 687 were fed winter feed rations and 1 125 received summer feed rations. Blood samples were taken from v. jugularis into heparinized tubes and at the same day the methemoglobin concentration was determined quantitatively by the method according to Podivínský (Homolka 1969). The determination was carried out photometrically as a per cent of the total hemoglobin. The error of the method did not exceed 0.9 % of the determined values.

The animals came mostly from the South-moravian region. At every agricultural farm a set consisting usually of 18 cows was examined; the latter was further divided into three groups of six animals: I - cows in the 1st lactation stage, 14 days - 2 or at most 3 months after parturition, II - cows in the 2nd lactation stage, 4-6 months post partum and III - cows in the 8th - 9.5 month of pregnancy, usually in the dry period. The results were statistically evaluated by the t-test and F-test from three aspects: 1) value differences between fresh and winter feeding, 2) differences among individual years with the same type of feed rations, and 3) differences among individual cow groups at the same feeding season.

At the time of blood sampling the feed ration of individual cow groups was found and the nutrient requirements were calculated to meet the Czechoslovak standard ČSN 46 7070; the calculated value was increased by 10 % to cover the losses in the basic feed ration. The calculation of the nutrients supplied was carried out according to laboratory analyses of feedstuffs. If the feeds were not analyzed, the average values known from the area (mostly of fresh grass and dry feedstuffs) or the values presented in the ČSN 46 7007 (e. g. of grain, straw and byproducts and residues of food industry) were taken as the basis. From the total number of 17 examined nutrients the energy nutrients (digestible nitrogen compounds and starch units), having the highest effect upon the rumen processes influencing the nitrate reduction, are presented here.

The length of vegetation periods given by the number of weeks, when fresh grass was fed, was in the individual year season (A, B, C, D) as follows: A - 27, B - 22, C - 21, D - 21 weeks.

The data obtained in this study have been used not only for the research purposes but also, according to the situation observed in individual agricultural farms, they were recommended to feeding, prevention, therapy and management practices.

Results

It follows from methemoglobinaemia distribution curves of the whole cow populations (Fig. 1) for the whole 4 years that the summer ($5.4 \% \pm 1.72$) and winter (5.37 ± 1.85 %) values, and their scatter as well, do not differ significantly. The Gaussian character with the peak slightly shifted to the left can be seen. The median of the winter values, 4.61 %, is lightly lower than that of summer values (4.676). The average values of the populations in both periods moderately exceed the toleration limit of 5 % of hemoglobin which is regarded in recent literature as the upper physiological limit (Spörri and Stünzi 1969; Jagoš and Bouda 1981).

No significant differences in methemoglobinaemia have been found among individual cow groups fed fresh grass. As far as the winter feed rations are concerned, the values of methemoglobinaemia in highly-pregnant cows were found to be signi-

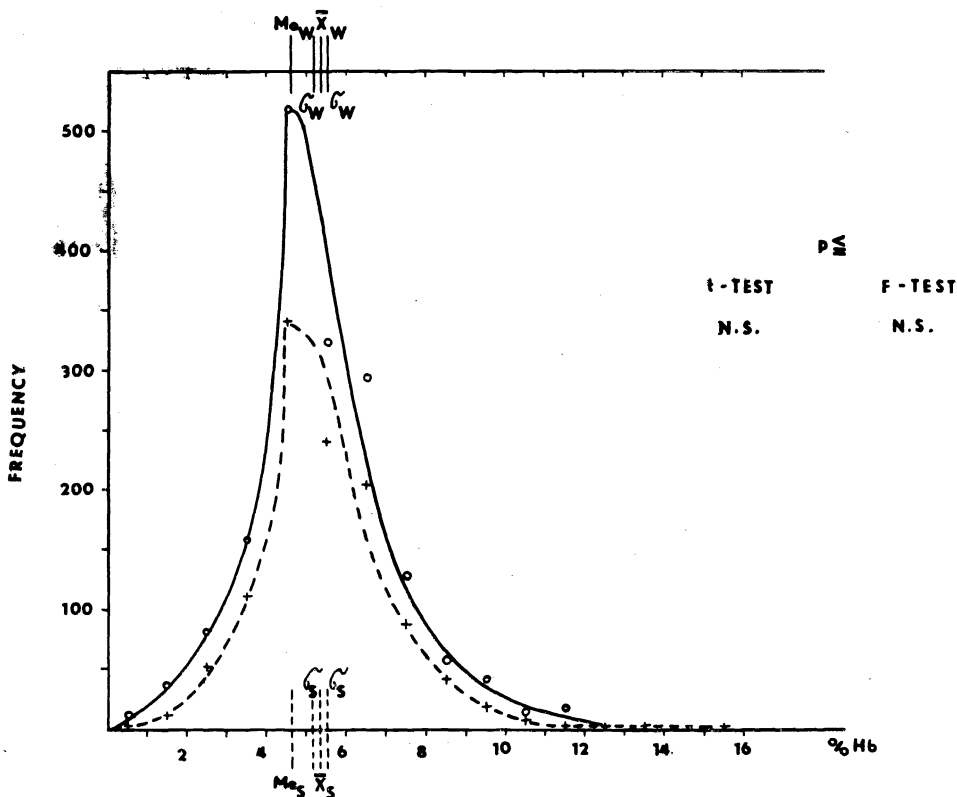


Fig. 1.

Distribution of methemoglobin values in cow populations for the whole 4-year period. Winter feed rations (solid line, rings, subscript W) and summer feed rations (dashed line, crosses, subscript S).

Average values (\bar{x}), median (Me) and standard deviations (σ).

ificantly lower than in the two remaining lactating groups (Fig. 2). This tendency was also non-significant in the summer period. The difference between summer and winter levels of methemoglobinaemia in the same cow groups and for the whole 4 years were determined not to be significant, the scatter being significantly higher in winter season in the group II and III.

The differences in feed rations of the individual cow groups according to lactation and reproduction stage manifested by the degree of methemoglobinaemia are differing in the winter period only. The feed composition and quality in individual years has, however, significantly influenced the methemoglobinaemia level in the period of fresh and winter feeding (Fig. 3) in whole populations and in individual groups (Fig. 6). The exception was observed in the case of the C/D period in the group I and III (Fig. 4 and 5) in summer and winter period. The highest winter values of methemoglobinaemia were found in the B period, whereas the summer values were highest in the A period. In the last two years of investigation a decrease of methemoglobinaemia has been observed in all cow groups, this being more pronounced in winter than in summer seasons. The differences between summer and winter

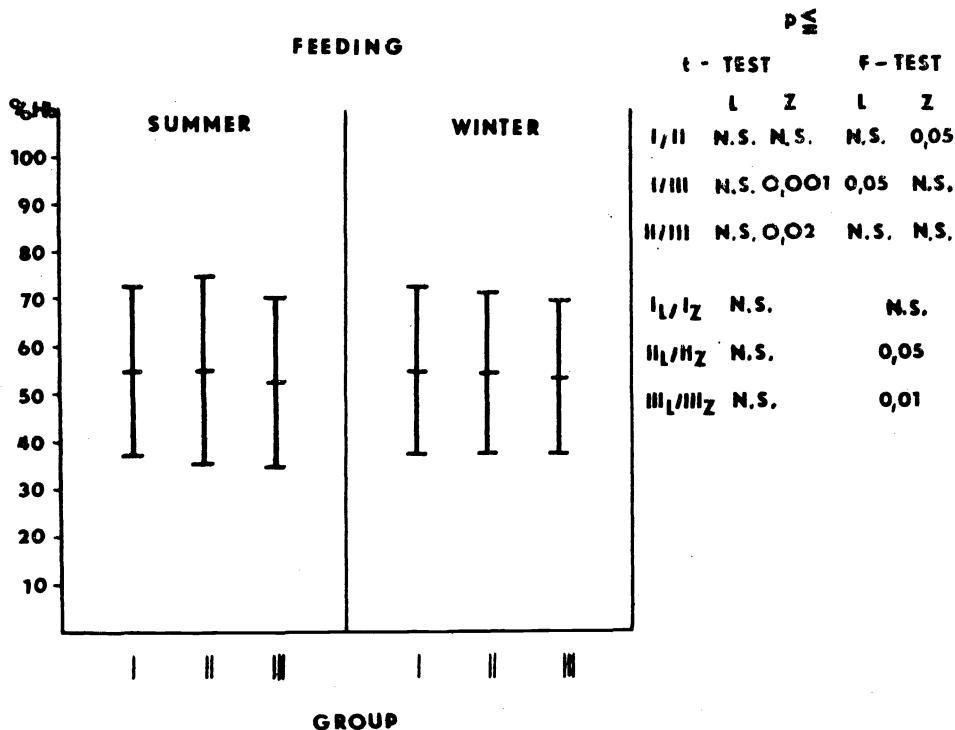


Fig. 2

Average values and standard deviations of methemoglobinaemia in groups of cows (I, II, III) for all four winter and summer feeding periods. (Winter feed rations = Z summer feed rations = L).

methemoglobin levels of the same cow groups were found to be significant in 40 % only. The highest average values in winter B period were observed in the group II (7.269 % \pm 1.47) whereas significantly lower values were found in the group III. The severest methemoglobinaemia in summer season was found again in the group II (6.153 % \pm 1.839) in the A period, the difference being however not significant when compared with the two remaining groups (Table 1). Generally, the differences in methemoglobinaemia among cow groups at the same feeding period were significant only in isolated cases (in 3 out of 24), i. e. at the lower level of significance than in the case of differences among individual years. A similar situation has been observed as far as the value scatter is concerned which differed in 25 % of cases only.

Table 2 shows that the methemoglobin concentrations exceeding the 5 % limit of tolerance were in all periods ranging mostly from 5 to 10 %, the frequency of values ranging from 10–15 % being quite low (5.92 % of cases at the most, in the group III in the A period and with winter feed rations). Concentrations exceeding 15 % of hemoglobin occurred only in two cows of the group I in summer season. The table corroborates the favourable development in the methemoglobinaemia incidence over the last two years, showing also the unfavourable situation in the summer season of the A period and subsequent winter B period. In this period exceptionally high frequency of moderate methemoglobinaemia in the interval 5–10 % affecting 95 % of cases was observed.

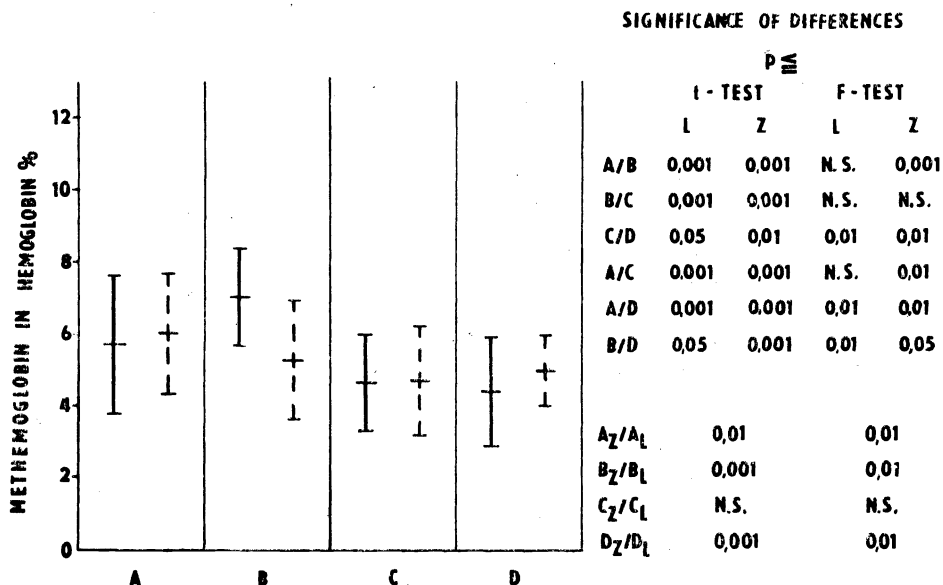


Fig. 3
Methemoglobinaemia in cow populations in individual year seasons (A, B, C, D). Denotation of winter and summer values is the same as in Fig. 1 and 2.

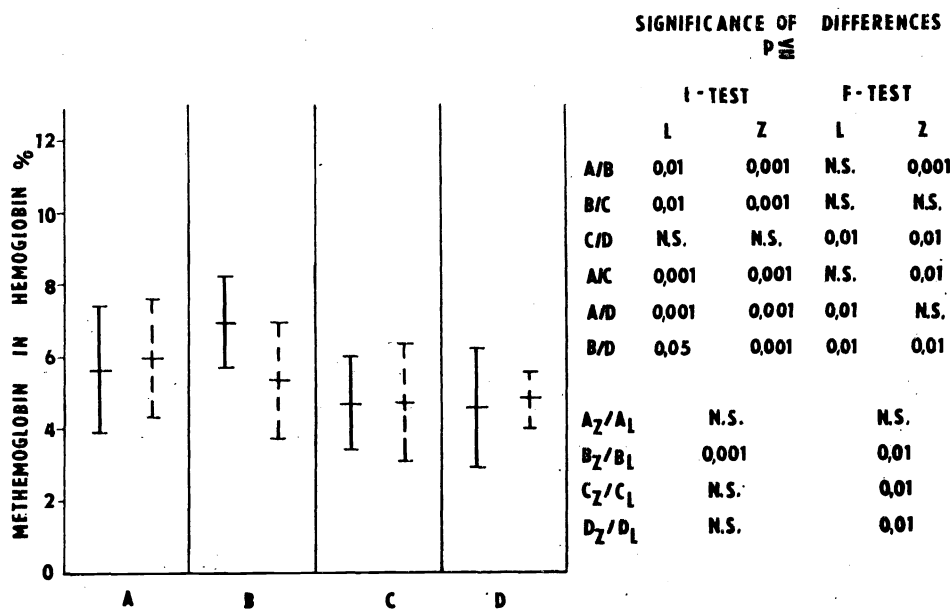


Fig. 4
Methemoglobinaemia in cows of the group I in individual year seasons.

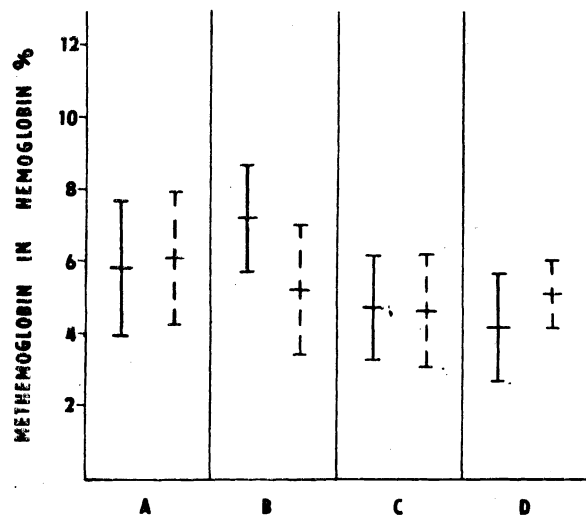


Fig. 5

Methemoglobinaemia in cows of the group II in individual year seasons.

SIGNIFICANCE OF DIFFERENCES

	P _{MA}			
	I-TEST		F-TEST	
	L	Z	L	Z
A/B	0,001	0,001	N.S.	0,01
B/C	0,05	0,001	N.S.	N.S.
C/D	0,05	0,01	0,01	N.S.
A/C	0,001	0,001	N.S.	0,01
A/D	0,001	0,001	0,01	0,01
B/D	N.S.	0,001	0,01	N.S.
A _Z /A _L	N.S.		N.S.	
B _Z /B _L	0,001		0,05	
C _Z /C _L	N.S.		N.S.	
D _Z /D _L	0,001		0,01	

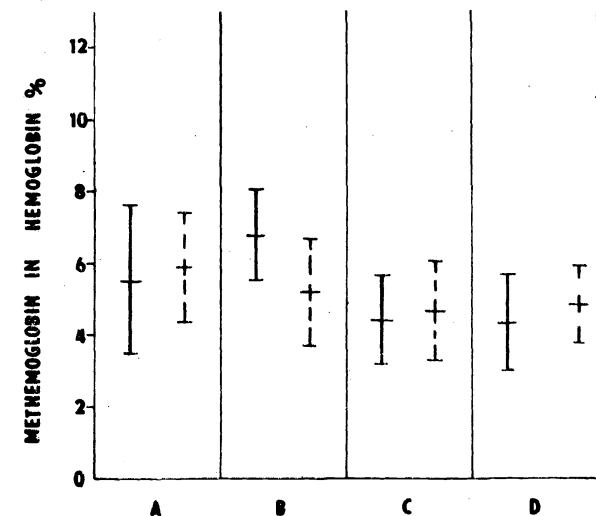


Fig. 6

Methemoglobinaemia in cows of the group III in individual year seasons.

SIGNIFICANCE OF DIFFERENCES

	P _{MA}			
	I-TEST		F-TEST	
	L	Z	L	Z
A/B	0,001	0,001	N.S.	0,01
B/C	0,02	0,001	N.S.	N.S.
C/D	N.S.	N.S.	0,05	N.S.
A/C	0,001	0,001	N.S.	0,01
A/D	0,001	0,001	0,01	0,01
B/D	N.S.	0,001	0,01	N.S.
A _Z /A _L	N.S.		0,01	
B _Z /B _L	0,001		0,05	
C _Z /C _L	N.S.		N.S.	
D _Z /D _L	0,02		0,05	

Table 1

Significance of differences of methemoglobinaemia values of the groups of cows in individual summer (S) and winter (W) feeding period during 4 years (A, B, C, D)

Pe-ri-od	Fee-ding	Methemoglobin in % of hemoglobin			Significance of differences $P \leq$ (N. S. = not significant)					
		I	II	III	I/II		I/III		II/III	
					t-test	F-test	t-test	F-test	t-test	F-test
A	W	5.675 ± 1.757	5.880 ± 1.864	5.513 ± 2.106	N. S.	N. S.	N. S.	0.05	N. S.	N. S.
	S	5.982 ± 1.639	6.153 ± 1.839	5.886 ± 1.523	N. S.	N. S.	N. S.	N. S.	N. S.	0.05
B	W	6.984 ± 1.263	7.269 ± 1.470	6.808 ± 1.263	N. S.	0.05	N. S.	N. S.	0.02	N. S.
	S	5.370 ± 1.612	5.276 ± 1.808	5.186 ± 1.489	N. S.	N. S.	N. S.	N. S.	N. S.	0.05
C	W	4.714 ± 1.291	4.790 ± 1.433	4.426 ± 1.244	N. S.	N. S.	N. S.	N. S.	0.05	0.05
	S	4.728 ± 1.617	4.710 ± 1.563	4.671 ± 1.395	N. S.	N. S.	N. S.	0.05	N. S.	N. S.
D	W	4.586 ± 1.657	4.258 ± 1.491	4.340 ± 1.342	N. S.	N. S.	N. S.	0.01	N. S.	N. S.
	S	4.867 ± 0.843	5.195 ± 0.933	4.860 ± 1.084	0.05	N. S.	N. S.	0.05	N. S.	N. S.

Table 2

Percentage of methemoglobinaemia values exceeding 5 % of hemoglobin in individual stages (A, B, C, D) and in individual groups of cows fed summer (S) and winter (W) feed rations

Period	Feeding	Methemoglobinaemia								
		> 5 %			> 10 %			> 15 %		
		I	II	III	I	II	III	I	II	III
A	W	55.84	63.29	50.66	2.60	3.80	5.92	0	0	0
	S	65.67	75.81	69.29	0.72	4.03	0	0	0	0
B	W	95.24	96.58	97.44	1.59	4.27	1.71	0	0	0
	S	52.78	45.79	42.86	0	1.87	0.95	0	0	0
C	W	47.69	34.35	32.74	1.69	0.76	0	0	0	0
	S	41.77	41.98	45.12	3.80	0	0	2.58	0	0
D	W	37.09	29.41	28.00	1.32	0	0	0	0	0
	S	35.00	47.46	48.00	0	0	2.44	0	0	0

Discussion

It follows quite clearly from the results that the highest effect upon the frequency and level of methemoglobinaemia in dairy cows could be ascribed to climatic conditions of the individual years, influencing probably the nitrate concentration in fresh

and preserved foodstuffs. These conditions appeared to exert their effect also by the portion of readily fermented carbohydrates contained in feeds. This portion influenced also the nutrient ration since in the periods with higher methemoglobinaemia levels higher urea concentrations in the cow blood were also observed (Lebeda and Buš 1984), confirming thus the reduced nutrient ratio. More severe methemoglobinaemia occurred in the years with a longer vegetation period when also a higher level of beta-carotinaemia appeared (Lebeda and Prikrylová 1983).

The theory that the methemoglobinaemia level is mostly influenced by basic feeds of a ration is corroborated also by the fact that the differences in methemoglobinaemia among individual cow groups at the same feeding periods are non-significant in 87.5 %. The effect of individual summer and winter feeding periods has been found to be more pronounced, the methemoglobinaemia degree differing in 41.67 % with higher levels occurring more often in summer than in winter. This is indicative of a higher nitrate content and a lower nutrient ratio in fresh grass. The winter season of the period B however demonstrates that after the period of fresh feeding, which is unfavourable from the point of view of methemoglobinaemia, even worse period of winter rations can follow. When comparing several years' averages, no significant differences have, however, been found between the degree of methemoglobinaemia in winter and summer feed rations.

The risk of chronic subclinical methemoglobinaemia is thus practically identical in both feeding periods. The reason of the occurrence of less pronounced methemoglobinaemia in high pregnant cows, occurring significantly in winter period and less markedly in summer period, has not yet been elucidated.

The observed levels of methemoglobinaemia do not yet induce clinical signs with the exception of mucosal cyanosis, which is appearing already at concentrations of 5–15 % of methemoglobin (Bartík et al. 1974). Demonstrated cases of methemoglobinaemia are, with the highest probability, chronic and it is thus necessary to take into account the fact that they decrease the milk production. This fall is caused by dissipating a part of the production energy of the feed ration for the compensation purposes.

The adverse effect of cold and wet weather upon the metabolism of plants and nitrate content in forage can also be confirmed by frequent incidence of methemoglobinaemia (reaching as much as 12 % of hemoglobin) in cows during the first half of the fresh grass feeding in 1984. This will be the subject of another paper.

The present work corroborates again our previous observation (Lebeda et al. 1978) that methemoglobinaemia of dairy cows in the South-moravian country district is of subclinical character. It exhibits the features typical for production diseases as shown by Payne (1972). Its negative effect can be seen first of all in the decrease of energy available for production purposes and in a combined effect with other nutritional deficiencies and metabolic disturbances.

The prevention of this disease lies thus not only in agrotechnical measures (adequate fertilization and application of herbicides, proper rationing of feeds with higher nitrate content, crop period regulation) but also in the adjustment of ration composition and feeding management. The latter measures include also the reducing the nitrate ill-effect upon the organism and inhibiting methemoglobinaemia development by offering a sufficient amount of easily digestible saccharides, beta-carotene and possibly of vitamin A and by providing facilities for a suitable and short-term storing of fresh grass in preparing rooms, etc.). Well-balanced feed rations corresponding to the animal requirements are also necessary. The reserves should be made preferably of carbohydrate feedstuffs, reaching 20 % of a yearly consumption, available for compensation of adverse situation in feed balance during individual years.

Úroveň methemoglobinémie dojnic v průběhu čtyř roků

V průběhu 4 roků (etapy A, B, C, D) byla zjištěna methemoglobinémie ve venózní krvi 2 812 krav (1 687 v období zimních krmných dávek, 1 125 v období zelených krmiv) v 1. a v 2. fázi laktace a v 8.—9,5. měsíci březosti. Průměrné hodnoty methemoglobinu (Mthb) mírně přesahovaly tolerovatelnou mez 5 % hemoglobinu. Mezi zimními a letními hodnotami Mthb nebyl signifikantní rozdíl ani u celých populací ani u jednotlivých skupin krav. Krávy vysokobřeží měly v zimním období signifikantně nižší Mthb než skupiny laktující. Rozdílnost krmných dávek jednotlivých skupin krav měla jen malý vliv na úroveň Mthb. Signifikantní vliv však měla skladba a kvalita krmiv v jednotlivých rocích. Signifikantně nejvyšší Mthb byl zaznamenán u všech skupin krav v letním období etapy A a v po něm následujícím zimním období etapy B. Nejvyšší koncentrace měla v obou těchto obdobích skupina krav v 2. fázi laktace (7,27 % \pm 1,47 v zimním a 6,15 % \pm 1,84 v letním období). V posledních dvou rocích (C a D) nastal pokles Mthb u všech skupin krav, který byl výraznější v zimních než v letních obdobích.

Je diskutován vliv subklinických chronických methemoglobinemií na zdravotní stav dojnic a na ekonomické ukazatele chovu.

Уровень метгемоглобинемии дойных коров в течение четырех лет

В течение четырех лет (этапы А, Б, В, Г) была установлена метгемоглобинемия в венозной крови 2 812 коров (1 687 в период зимнего кормового рациона, 1 125 в период зеленых кормов) и на 1 и 2 фазе лактации и на 8—9,5 месяце беременности. Средние величины метгемоглобина (Mthb) незначительно превышали допустимый предел 5 % гемоглобина. В промежутке между зимними и летними величинами Mthb не было значимой разницы у всей популяции, ни у отдельных групп коров. Коровы на высокой стадии беременности отличались в зимний период значимо более низким Mthb чем лактирующие группы. Разница в кормовых рационах отдельных групп коров оказывала лишь незначительное влияние на уровень Mthb. Значительное влияние оказывал состав и качество кормов по отдельным годам. Самый большой уровень Mthb был установлен у всех групп коров в летний период этапа А и после него следующий зимний период этапа Б. Самой большой концентрацией в приведенные два периода отличалась группа коров на 2 фазе лактации (7,27 % \pm 1,47 в зимний период и 6,15 % \pm 1,84 в летний период). В течение последних двух лет (В и Г) наступает понижение Mthb у всех групп коров, которое было более выразительным в зимний чем в летний периоды.

Обсуждается влияние субклинических хронических метгемоглобинемий на состояние здоровья дойных коров и экономические показатели разведения.

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