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Research Institute of Experimental Veterinary Medicine, Košice, Slovak Republic[1]
University of Veterinary Medicine, Košice, Slovak Republic[2]

ANNA JACKOVÁ,[1] PAVOL SIKLENKA,[1] and JÁN PLEVA[2]

# Transrenal passage of nitrates and nitrites in calves (short communication)

## Summary

In a study with 12 calves on milk nutrition, the course of methemoglobinemia as well as transrenal passage of nitrates and nitrites after single *per os* administrations of 4 g $NaNO_2$ per animal and 30 g $KNO_3$ per animal in the form of water solutions has been observed. The response of the organism of calves to *per os* administered doses of sodium nitrite and potassium nitrate was observed by the determination of the methemoglobin percentage in blood and the nitrate and nitrite content in urine before the administration of the respective dose and after h 1, 2, 3 and 4 after the administration. A significant elevation in the values of methemoglobin was recorded after h 2 after the administration of 4g $NaNO_3$ per animal. The mean value of methemoglobin in blood was 18.84% of total hemoglobin. A slight decline in the values occurred as early as after h 3 after the administration. Of clinical signs, cyanosis of visible mucosae was observed. The highest nitrite and nitrate values in urine were determined after h 2 after *per os* administration of 4g $NaNO_2$. With the administration of 30g $KNO_3$ per animal, the most pronounced elevation in methemoglobinemia was observed after h 3, when the means values of methemoglobin was 11,75%. Of clinical signs, only slight cyanosis of mucosae was detectable. Maximum values of nitrates in urine of experimental calves after h 3 after the administration of 30 g $KNO_3$ per animal, with the mean value of 29,9 mM $NO_3^{-1}$ clearly demonstrate a good transrenal passage of nitrates in calves on milk nutrition.

Key words: calves, blood, methemoglobin, urine, nitrates, nitrites

## Zusammenfassung

Titel der Arbeit: **Nierenpassage von Nitraten und Nitriten bei Kälbern** (Kurzmitteilung)
Bei 12 Kälbern wurde während der Milchernährungsperiode der Verlauf der Methämoglobinämie und die Nierenpassage der Nitrate und Nitrite nach einmaliger peroraler Applikation von 4g $NaNO_2$ und 30g $KNO_3$, welche in wasserlöslicher Form verabreicht wurden, untersucht. Die Reaktionen der Kälber auf die perorale Applikation von $NaNO_2$ und $KNO_3$ wurden durch die Bestimmung des Methämoglobinprozentes im Blut und des Nitrat-und Nitritgehaltes im Urin vor der Applikation sowie 1, 2, 3 und 4 Stunden nach der Applikation verfolgt. Es wurde ein deutlicher Anstieg des Methämoglobins 2 Stunden nach der Applikation von 4g $NaNO_2$ je Tier beobachtet. Er betrug zu diesem Zeitpunkt durchschnittlich 18,84% vom Gesamthämoglobin. Ein geringer Rückgang der Werte stellte sich schon nach der 3. Stunde ein. Von den klinischen Symptomen wurde Zyanose der äußeren Schleimhäute festgestellt. Die höchsten Nitrat- und Nitritwerte ergaben sich 2 Stunden nach der peroralen Applikation von 4g $NaNO_2$. Bei der Applikation von 30g $KNO_3$ je Tier wurde ein erheblicher Anstieg der Methämoglobinämie ab der 3. Stunde ermittelt. Zu diesem Zeitpunkt erreichten die Methämoglobinwerte 11,75%. Von den klinischen Symptomen wurde nur einen leichte Zyanose der Schleimhäute beobachtet. Die höchsten Nitratwerte im Urin der Versuchkälbergruppe 3 Stunden nach der Applikation von 30g $KNO_3$ je Tier bei Durchschnittswerten von 29,9 mM $NO_3^{-1}$ beweisen eine gute transrenale Passage der Nitrate bei Kälbern während der Milchernährungsperiode.

Schlüsselwörter: Kälber, Nitrate, Nitrite, Methämoglobin, Blut, Urin

## Introduction

With the intake of feed and water, nitrates become a part of biochemical reactions in the organism. The course of these reactions is considerably influenced by anatomical and physiological distinctions of the digestive apparatus of individual farm animal species. In monogastric animals, nitrates are excreted from the organism mainly by kidneys. In a lesser extent, the reduction of nitrates takes part also in some organs, particularly in liver (BARTÍK and ROSÍVAL, 1971).

The nature of biochemical reactions of $NO_3^{-1}$ in the digestive tract of ruminants depends particularly on the amount of received nitrates, on the population of rumen microflora as well as on its enzymatic apparatus and on pH of both rumen and gut of animals (SKŘIVÁNEK, 1987). Nitrates are reduced in the digestive tract through oxygenous metabolites to ammonia. Mainly Vibrio succinogenes and Desulfovibrio desulfuricans participate in this activity. Rumen bacteria are capable of reducing as much as 65 mmol $NO_3^{-1}$ of rumen fluid to ammonia in the course of 2 to 3 hours. When the concentration of nitrates exceeds the limit of 65 mmol $NO_3^{-1}$, nitrates causing an intoxication of the animal begin to accumulate in rumen (BARTOŠ, 1987). The toxicity of nitrites consists in their direct reactions with hemoglobin, blood colouring matter, changing its ferro-form $Fe^{2+}$ to ferri- form $Fe^{3+}$, so-called methemoglobin (MtHb) that is not capable of reversible oxygenation (BARTÍK, 1968, ARCHER, 1982, OPLIŠTIL, 1985). In calves on milk nutrition, microbial reduction does not take part in such an extent as in adult ruminants; the enzymatic digestion prevails. The more intensive activity of forestomachs occurs in connection with their development, approximately on weeks 5-6, that is given by the system of feeding, particularly by the intake of solid feeds (VESELÝ, 1988).

## Materials and Methods

12 heads of Slovak spotted bullocks were included into the experiment during the time of milk nutrition. Calves were divided into experimental and control groups; each group consisted of six animals. The animals were included into experiment on day 3 after birth; the average live weight was 40 kg. Calves of experimental and control group were fed in the same way. Up to the age of 5 days, all calves were fed colostrum from their mothers, afterwards they were fed milk feed mixture of Laktavit. Laktavit is commercial product. From month 1 of the age onwards, both groups were fed alternatively Laktavit and milk supplemented with formic acid. Water and hay were provided *ad libitum*.

In the first part of the experiment, throughout 4 weeks, the experimental group was administered potassium nitrate in increasing doses per os (1 - 10 g of $KNO_3$ per animal and day) in the form of water solution in order to develop the adaptation to nitrate in these calves.

In the second part of model experiment, the administered dose of 10 g $KNO_3$ per animal and day was reduced, in experimental calves 1, 2, 3 to 2 g $KNO_3$ per animal and day, in experimental calves 4, 5, 6 to 5 g $KNO_3$ per animal and day. During the

administration of the above-mentioned doses on week 6 of the model experiment when the average live weight of calves was 63 kg, experimental calves 1, 2, 3 were administered *per os* a single dose of 4 g $NaNO_2$ per animal in the form of water solution. At the same time, experimental calves 4, 5, 6 were administered *per os* a single dose of 30g $KNO_3$ per animal in a water solution. The response of the organism of calves to *per os* administered sodium nitrite and potassium nitrate was observed by the determination of the percentage of methemoglobin of total hemoglobin before the administration, after h 1, 2, 3, and 4 after the administration of the respective dose. Blood was taken from *v. jugularis*. Blood was processed immediately after collection by the modified photometric method of HOMOLKA (1969).

In the course of model experiment, transrenal passage of nitrates and nitrites in experimental and control group of calves has been observed as well. Urine was stored at 4°C and processed on next day after the collection by the modified method of CVAK et al. (1985).

## Results and Discussion

The nitrite and nitrate content in colostrum, dried feed mixture of Laktavit, milk and water used to prepare Laktavit and for watering varied below the limit value. Therefore, there was no burden of the organism of calves by the excessive supply of nitrates and nitrites from feed and water. YONG et al. (1990) state that water was a source of nitrate intoxication in cattle. The milk of dairy cows cannot be the source of nitrates, with the exception of the excessive nitrate amount from the feed (BARANOVÁ et al., 1993).

Table 1 presents the concentration of methemoglobin of total hemoglobin in blood as well as nitrate and nitrite content in the urine of control calves. The mean MtHb values in blood are in accordance with the data of BARTÍK and ROSÍVAL (1971) who report the values of 0.3 - 1.7% MtHb of total hemoglobin as normal in the blood of ruminants.

Table 1
Concentrations of MtHb in blood and of $NO_3^-$ and $NO_2^-$ in urine of control calves (means SD, 6 calves per treatment) (Methämoglobin im Blut und $NO_3^-$ und $NO_2^-$ im Urin bei Kälbern der Versuchsgruppe (Mittelwert SD. 6 Kälber im Versuch))

| Day of the experiment | Blood MtHb % | Urine $NO_3^-$ Mm | Urine $NO_2^-$ mM |
|---|---|---|---|
| 1. | 1.61 ± 0.35 | 0.076 ± 0.003 | 0.005 ± 0.001 |
| 10. | 1.64 ± 0.39 | 0.077 ± 0.002 | 0.005 ± 0.002 |
| 20. | 1.65 ± 0.40 | 0.077 ± 0.001 | 0.006 ± 0.004 |
| 40. | 1.59 ± 0.39 | 0.078 ± 0.004 | 0.006 ± 0.001 |

Table 2 presents the concentration of methemoglobin of total hemoglobin in blood of experimental calves as recorded before the single per os administration of 4 g $NaNO_2$ per animal and after h 1, 2, 3, and 4 after the administration. A significant elevation in the values of methemoglobin occurred after h 2 after the administration. A slight

Table 2
Concentrations of MtHb in blood and of $NO_3^-$ and $NO_2^-$ in urine of calves given a single dosis of either 4 g $NaNO_2$ or 30 g $KNO_3$ (means SD, 6 calves per treatment) (Methämoglobinwerte im Blut und $NO_3^-$ und $NO_2^-$ im Urin bei Kälbern nach der einmaligen Applikation von 4 g $NaNO_2$ und 30 g $KNO_3$ (Mittelwert SD, 6 Kälber im Versuch))

| Time after dosing h | 4 g $NaNO_2$ Blood MtHb % | Urine $NO_3^-$ mM | Urine $NO_2^-$ mM | 30 g $KNO_3$ Blood MtHb % | Urine $NO_3^-$ mM | Urine $NO_2^-$ mM |
|---|---|---|---|---|---|---|
| 0 | 0,99 ± 0.39 | 1.9 ± 0.2 | 0.019 ± 0.007 | 1.69 ± 0.53 | 2.1 ± 0.3 | 0.012 ± 0.003 |
| 1 | 15.04 ± 0.48 | 2.6 ± 0.2 | 0.103 ± 0.006 | 3.09 ± 0.55 | 12.9 ± 0.2 | 0.090 ± 0.013 |
| 2 | 18.84 ± 0.93 | 3.4 ± 0.2 | 0.113 ± 0.003 | 7.03 ± 2.63 | 17.4 ± 0.9 | 0.094 ± 0.038 |
| 3 | 16.81 ± 1.94 | 2.0 ± 0.4 | 0.086 ± 0.024 | 11.75 ± 1.82 | 29.9 ± 1.2 | 0.099 ± 0.028 |
| 4 | 12.09 ± 3.60 | 1.7 ± 0.5 | 0.070 ± 0.020 | 9.89 ± 0.75 | 29.7 ± 1.3 | 0.091 ± 0.022 |

decline in the values of methemoglobin occurred as early as after h 3 after the administration. Of clinical signs of methemoglobinemia, only cyanosis of visible mucosae was observed. A marked cyanosis of visible mucosae was described by PISKAČ and KAČMÁR (1985) already with values of 5-15% MtHb of total hemoglobin, SLANINA (1985) with 15% MtHb in blood, NAGY et al. (1997) registered mild cyanosis at 20% MtHb, an expressive cyanosis at atainment of 35-40% MtHb of total hemoglobin.

Concentrations of the nitrate and nitrite in urine of experimental calves that were administered 4 g $NaNO_2$ per os are presented in Table 2. The highest values of nitrates and nitrites in urine were after h 2 after the administration of 4 g of sodium nitrite.

BARTÍK and ROSÍVAL (1971) report lethal doses for small ruminants as follows: 100 mg $NaNO_2$/ kg l.w. and 30 g $KNO_3$/ total body weight of the animal. Mean values of methemoglobin in blood of experimenal calves recorded before the single per os administration of 30 g $KNO_3$ per animal and after h 1, 2, 3, and 4 after the administration are presented in Table 2. Maximum value of methemoglobin in blood - 11,75 % of total hemoglobin were observed after h 3 after the administration. Even though these MtHb values exceed the tolerable level of 5%, according to LEBEDA and PŘIKRYLOVÁ (1985) only slight cyanosis of mucosae was seen in experimental calves. The results of this experiment are in accordance with the data of BOUDA et al. (1986) who recorded an elevation of methemoglobin in blood to 10-12.6% after single daily dose of 18 g $NO_3$ per os in 8-week-old calves on milk nutrition.

The nitrate and nitrite content in urine of experimental calves before the administration and after h 1, 2, 3, and 4 after single per os administration of 30 g $KNO_3$ per animal is presented in Table 2. Maximum values were recorded after h 3 after the administration. The mean value of nitrates in urine of 29,9 mM $NO_3^{-1}$ clearly demonstrates the transrenal passage of nitrates in calves on milk nutrition that is in accordance with the data of BARTÍK and ROSÍVAL (1971) as well as SLANINA et al. (1990).

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Authors addresses

MVDr. ANNA JACKOVÁ, Csc.;
MVDr. PAVOL SIKLENKA, CSc.
Research Institute of Experimental Veterinary Medicine
Hlinkova 1/A
040 00 Košice
Slovak Republic

Prof. MVDr. JÁN PLEVA, Dr.Sc.
University of Veterinary Medicine
Komenského 73
040 00 Košice
Slovak Republic

E-Mail: skalicka@linux.vuvm.sk

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## Buchbesprechung

**Die Warmblutzucht in Mecklenburg - Vorpommern**

OTTFRIED WEIHER

120 Seiten, 31 Farbfotos, 105 s/w Fotos, 53 s/w Graphiken, 7 Tabellen, Edition Temmen, Bremen, Rostock, 1998, ISBN 3-86108-720-0, 48,00 DM, 350,00 ÖS, 46,00 SFr

Die Pferdezucht fesselte und begeisterte in allen Jahrhunderten viele Menschen.
Nicht nur auf passionierte Züchter wirkt das Pferd wie ein Magnet. Es ist für die meisten naturverbundenen Menschen ein Sinnbild für schwebende Eleganz, für gezähmte Kraft und liebenswürdige Sanftmut. Das Pferd war aber immer auch von großer wirtschaftlicher Bedeutung. In der Landwirtschaft, im Transportwesen, für das Militär und den Sport war es unersetzlich. Mit der Entwicklung der Produktionsmittel und Produktivkräfte veränderten sich die Anforderungen an das Pferd und dementsprechend die Zuchtziele. Dieser Wechsel war besonders gravierend im 20. Jahrhundert. Dem engagierten Autor ist es gelungen, ein eindrucksvolles Bild der Entwicklung der Mecklenburgischen Warmblutzucht von der Antike bis zur Neuzeit zu zeichnen. Da Maler und Bildhauer aller Jahrhunderte das Pferd immer wieder meisterhaft gestaltet haben, konnte er mit sehr schönen Bildern und spannenden Legenden eine packende Geschichte erzählen. Das Buch enthält umfassende Informationen über herausragende Pferde und Menschen im Wandel der Zeit genauso wie über die Entwicklung der Züchtung und der sie bestimmenden gesellschaftlichen Verhältnisse.
Im Einzelnen gliedert sich das Buch in 7 Abschnitte mit folgenden Inhalten: Blick in die Geschichte (Antike), Ursprung des echten Mecklenburgers, Blütezeit der Mecklenburger Pferdezucht (im 18. Jahrhundert), Niedergang und Neubeginn im 19. Jahrhundert, Jahrhundertwende bis zweiter Weltkrieg, Zwischen Kriegsende und 1990, sowie ein kurzer Ausblick.
Den Abschluß des Buches bilden ein Literaturverzeichnis und ein Register.
Das Buch ist übersichtlich gegliedert, reich illustriert, durch Legenden um berühmte Pferde und Menschen immer wieder interessant gestaltet und mit zahlreichen Zeitzeugnissen eine ausgezeichnete Quelle für Pferdefreunde und Pferdezüchter, nicht nur in Mecklenburg - Vorpommern. Es vermittelt einen sehr guten Überblick über die geschichtlichen Wurzeln einer Pferdezucht, die heute international bekannt und bedeutungsvoll ist. Dieses Buch ist allen Freunden des Pferdes, den Reitern, den Züchtern, den Studenten und Lehrkräften sehr zu empfehlen. Wer die Zukunft gestalten will, soll die Vergangenheit kennen. Das Buch ist dafür eine große Hilfe.

HARTMUT FRANZ, Dummerstorf