

SYNDROME OF CEREBROCORTICAL NECROSIS EXPERIMENTALLY INDUCED IN CALVES BY ADMINISTRATION OF AMPROLIUM

J. BOUDA, P. JAGOŠ, V. DVOŘÁK, L. PIVNÍK

Department of Diagnostics, Therapy and Prevention of Animal Diseases, University of Veterinary
Science, 612 42 Brno

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Abstract

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In the experiment into which three experimental and four control calves were included the syndrome of cerebrocortical necrosis (CCN) was induced by the administration of Amprolium. Pathogenesis and symptomatology of the condition were followed. After the intraruminal administration of Amprolium (0.5g/calf/day) the clinical signs appeared in two calves after six weeks while in the third calf after 56 days. The main clinical features were: inappetence, blindness, increased excitability, symptoms of static and kinetic ataxia. Calves rose with difficulties, had difficulty avoiding objects, muscular tremor and opisthotonus appeared, when falling tonic-clonic convulsions and paddling movements were observed. The observed clinical signs were identical with those of spontaneous cases of CCN in calves.

Statistically significant decrease of thiamine content was determined in cerebral cortex, cardiac and skeletal muscle of experimental calves. In liver tissue there was no significant decrease of thiamine level. Fourteen days after the administration of Amprolium the erythrocyte transketolase activity was found to decrease while the thiamine pyrophosphate effect in blood increased. Pathologico-anatomical examination of brain revealed cerebral oedema and yellow coloured foci on some gyri. Histopathological changes in these parts were characterized by necrosis of the grey matter and enormous infiltration of macrophages. However, when comparing the plasma levels of vitamin A, E and C, minerals, Na, K, Ca, Mg, inorganic P, Zn, Cu, or urea, glucose, aspartateaminotransferase and gamma glutamyl transferase activity, acid-base balance in the blood and of some parameters in the rumen fluid, no significant differences were found between experimental and control groups.

Calf, plasma, hypovitaminosis B₁, transketolase, TPP effect, vitamins A, E, C, minerals Na, K, Mg, Ca, inorg. P, Zn, and Cu, urea, glucose, acid-base balance, rumen fluid.

On the basis of knowledge obtained in traditional forms of husbandry it was assumed that the vitamin B₁ requirement in ruminants was fully covered by microbial synthesis in forestomachs. Changes in husbandry practices particularly in feeding and dietetic practices, have corrected some of the common opinions concerning above all the rearing of calves and young cattle, including beef cattle. Calves in their first weeks of life are entirely dependent on thiamine supply from feed (Draper, Johnson 1951; Benevenga et al. 1964). Milk and colostrum contain large quantities of thiamine. Cow colostrum contains 60 mg/l, milk 30 mg/l (Zintzen 1973). It is apparent from

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literature data that the thiamine requirement in ruminants is dependent on animal age, feeding, quality and type of diet and on other factors as housing, infection or parasitic invasion.

The thiamine deficiency is caused by its insufficient dietary supply or by the action of a thiamine antagonist. Thiamine antagonists occur in various plants, bacteria and fungi. They can change the chemical structure of vitamin B₁, or their own chemical structure closely resembles that of thiamine. The action of these antagonists in enzyme systems is caused by the mechanism of competitive inhibition. The significant role in the incidence of hypovitaminosis B₁ in ruminants is ascribed to thiaminases which decompose vitamin B₁ (Shreeve, Edwin 1974; Morgan, Lawson 1974).

One of the causes of the cerebrocortical necrosis (CCN) syndrome could be due to the decreased synthesis of vitamin B₁ in the rumen occurring in digestive disturbances in forestomachs. The syndrome of cerebrocortical necrosis is often linked with rumen acidosis (Flachowsky et al. 1974; Jagoš et al. 1979).

The CCN syndrome can be induced experimentally by thiamine antagonists, such as pyrithiamine, oxythiamine and amprolium. Out of these the most suitable preparation for the experiments seems to be a coccidiostat Amprolium (Pill et al. 1966; Little 1969; Espinasse et al. 1971; Loew, Dunlop 1972; Lilja 1973; Markson et al. 1972, 1974).

A sporadic occurrence of CNS disease in calves and young cattle with clinical symptoms resembling those of CCN has already been observed in various parts of Czechoslovakia. In recent years this syndrome has been appearing more frequently, particularly in older calves (Jagoš et al. 1981). The purpose of the present work was to study the manifestation of hypovitaminosis B₁ in calves. Our attention was focused at a deeper elucidation of the genesis of this disease, at clinical, biochemical and pathologico-morphological symptoms of experimentally induced CCN syndrom as well as at the elaboration of objective diagnostic methods in relation to a spontaneous incidence of this disease.

Materials and Methods

Seven calves of the Bohemian Pied Breed were included in the experiment. Three experimental calves were 3 months old, their mean body mass at the beginning of the experiment being 97 kg. The control group consisted of four calves with mean body mass of 95 kg. Prior to the Amprolium administration the experimental and control calves underwent a three week adaptation period during which the animals were changed to a defined type of feed ration. The diet consisted of granulated feeds, alfalfa hay, straw and water. The CCN syndrome was induced by the daily administration of Amprolium (0.5 g/kg, later 0.75 g/kg of body mass). The total time period of the administration of Amprolium was 42–54 days.

The clinical examination of calves was carried out daily and included the determination of respiratory and pulse rate, temperature, the examination of alimentary tract, respiratory, cardiovascular, musculoskeletal and nervous systems. When first signs appeared, reflexes, cutaneous and subcutaneous perceptivity and musculoskeletal systems were examined twice a day. Daily body mass gains amounting to 0.61 kg/head and 0.75 kg/head of experimental and control group, resp., were followed.

Blood samples were collected from v. jugularis. During the experiment the rumen fluid was taken six times using a stomach tube. Samples of blood, rumen fluid and urine both from experimental and control animals were collected after killing. From each calf three samples of cerebral cortex, a sample of cardiac muscle from the left ventricle, of liver, and of striated muscle (m. gracilis) were taken.

Thiamine level in tissues was determined by the method of Picková (1969). Transketolase (TK) activity and thiamine pyrophosphate (TPP) effect were measured by a modified method by Brin (1974). The determination of vitamin A and E in blood plasma was carried out using a Perkin-Elmer 204 spectrofluorometer, by the method of Thomson et al. (1971, 1973) as modified by Jagoš et al. (1978). Concentration of vitamin C was assayed by a method described by Bessey et al. (1947).

Acid-base balance of venous blood was determined by the Astrup method on a Blood Micro-Equipment (BME 22) Radiometer using the Siggaard-Andersen nomogram. Hematocrit was determined by a routine micromethod in capillaries, hemoglobin and leucocyte count using the Coulter Counter. Concentrations of Na, K, Ca, Mg, P, Cu and Zn in blood plasma were determined by atomic absorption spectrophotometry. Inorganic phosphorus, aspartate aminotransferase (AST) and gamma glutamyl transferase (GMT) activities, levels of glucose and urea in blood plasma were obtained using the Bio-tests (Lachema Brno). In rumen fluid the determination of pH, acetic, propionic and butyric acids were carried out by gas chromatography.

Results

The food consumption of the Amprolium-treated calves was normal until the development of clinical signs. Four to five weeks after the administration of Amprolium bristled hair on head and back of the experimental animals were noted. Body temperature, heart and respiratory rates were normal. After six weeks following the administration a slight inappetence, increased excitability, gradual blindness, symptoms of static and kinetic ataxia appeared. Calves rose with effort, had difficulty avoiding objects, muscle tremor, opisthotonus and when falling tonic-clonic convulsions developed. Convulsions were accompanied by accelerated respiratory and higher pulse rate, general weakness and recumbency. In recumbent calves tonic-clonic convulsions lasting 1–3 minutes and paddling were also observed. It was possible to induce tonic-clonic convulsions by sound and light stimuli. On both hind limbs and on fore limbs distally from carpus the cutaneous sensitivity disappeared. In recumbent animals marked disturbances of digestive system characterized by inappetence, tympany and diarrhoea were found. As for the third calf the same clinical signs were manifested 54 days after the administration of Amprolium.

In order to find objectively the primary pathologicomorphological changes on CNS the calves were slaughtered 1–2 days after becoming recumbent. Pathologico-anatomical as well as histological examination revealed yellow discoloration of some cortical gyri. Histological changes in these parts were characterized by necrosis of the grey matter and infiltration of macrophages.

The thiamine content in cerebral cortex and skeletal muscle of the experimental group was found to be significantly lower ($P < 0,01$) when compared with the control group. Statistically significant decrease ($P < 0.05$) of thiamine content was determined also in heart muscle (Fig. 1). A decreased thiamine content was found even in liver tissue of experimental animals but this was, however, not of statistical significance in comparison with the controls. Erythrocyte transketolase (TK) activity and thiamine pyrophosphate (TPP) effect of experimental and control calves during

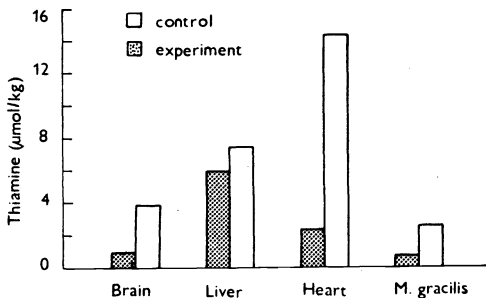


Fig. 1

Thiamine content in brain liver, heart and skeletal muscle of experimental (Amprolium-treated) and control group of calves

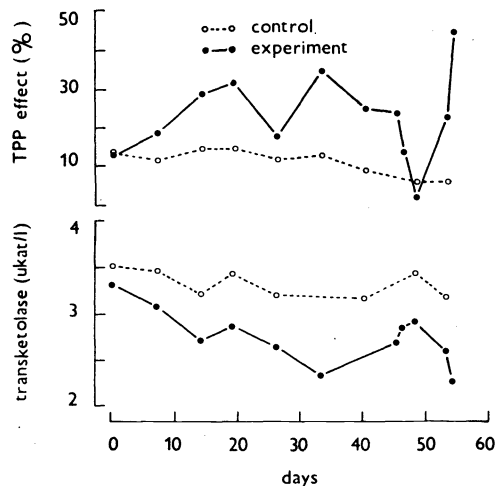


Fig. 2

Thiamine pyrophosphate effect and erythrocyte transketolase activity of calves

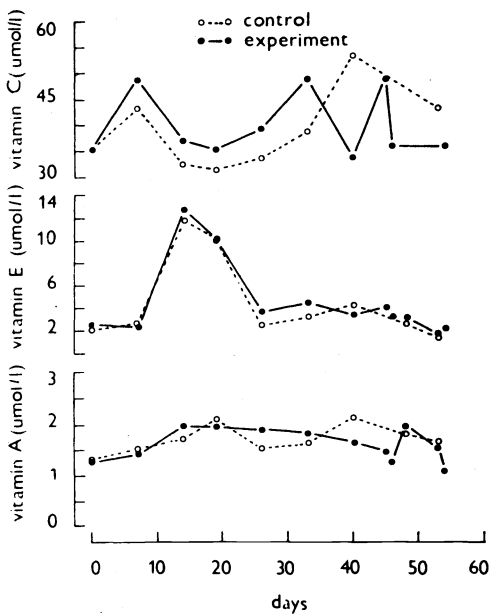


Fig. 3
Vitamin A, E and C levels in blood plasma of calves

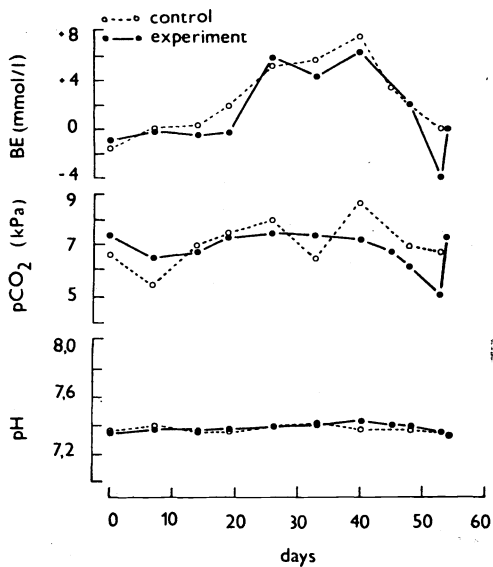


Fig. 4
pH, base excess, pCO₂ in blood of calves

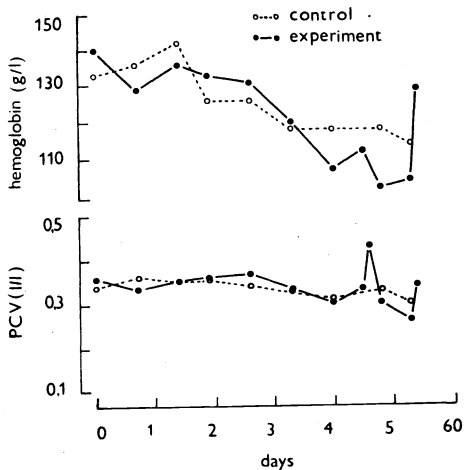


Fig. 5
Hemoglobin concentration and PCV values in blood of calves

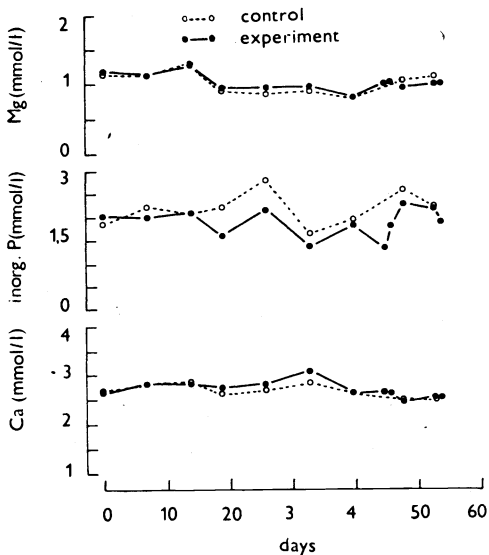


Fig. 6
Ca, inorganic P and Mg levels in blood plasma of calves

the comparable period statistically significantly different already one month after the Amprolium administration (Fig. 2) As for control animals the transketolase activity and TPP effect were found to be lower.

The results of vitamin A, E and C determination in blood plasma of calves are presented in Fig. 3. During the experiment a significant increase in vitamin A and E concentrations in blood plasma of the experimental group was observed only once.

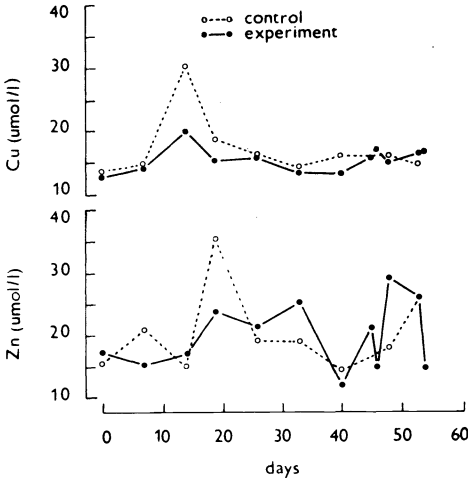


Fig. 7

Zn and Cu levels in blood plasma of calves

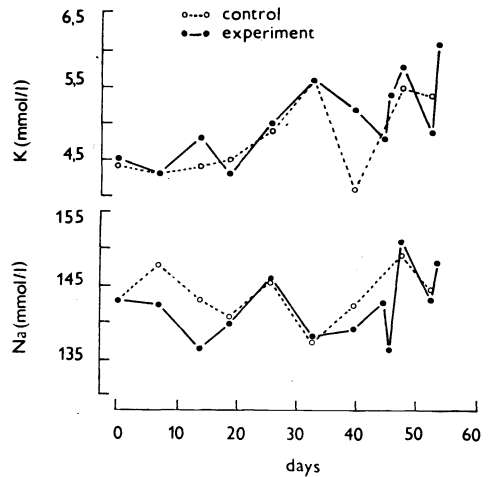


Fig. 8

Na and K concentrations in blood plasma of calves

Fig. 4 shows the dynamics of acid-base balance changes in the blood of calves. In the course of the experiment non-significant differences were found between both groups of calves. At the beginning a tendency to a slight acidosis was established in the blood of both groups, later the base-excess (BE) values increased to alkaline range which indicated the development of alkalosis. BE values dropped however again after the manifestation of clinical signs. As for PCV and hemoglobin values in blood (Fig. 5), plasma concentrations of Ca, inorganic P, Mg (Fig. 6), Zn and Cu (Fig. 7), Na and K (Fig. 8), urea, glucose and total protein (Fig. 9) no significant differences were found between both groups of calves. Between AST and GMT activities in blood plasma of experimental and control groups repeatedly no significant differences were established, the exception being the increase of AST activity (Fig. 10) in recumbent calves of the experimental group. One month after the onset of the experiment significant lower content of acetic acid and total content of volatile fatty acids in rumen fluid was observed in one case in the experimental group, the content of butyric acid being, however, increased.

Discussion

Amprolium, one of the thiamine antagonists which can induce the thiamin deficiency, was used in our experiment, the reason being that according to Markson (1972) the histopathological changes induced by the administration of this antimetabolite in central nervous system of young calves resemble very much the changes developed in spontaneous cerebrocortical necrosis. The origin of these changes could be explained by the action of Amprolium that blocks the ac-

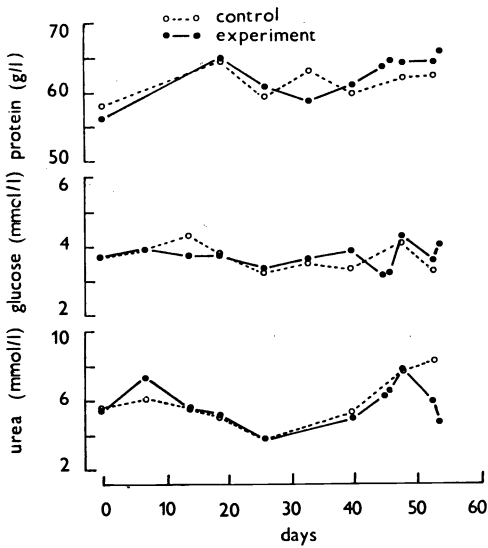


Fig. 9

Urea, glucose and protein concentrations in blood plasma of calves

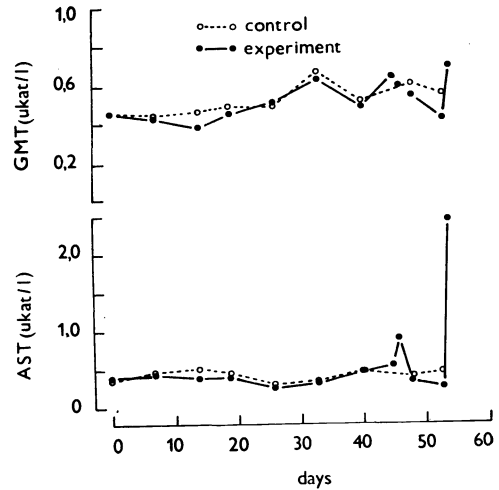


Fig. 10

Aspartate aminotransferase and gamma glutamyltransferase activity in blood plasma of calves

tive transport of thiamine, probably by competition on membranes (Sharma and Quastel 1965).

Clinical signs experimentally produced in calves by the administration of Amprolium were very similar to those of spontaneously diseased calves with CCN. At present the knowledge of ethiopathogenesis of CCN is not complete and a more detailed research into this problem is needed, particularly in the field concerning the interactions of metabolites.

The results of laboratory analyses of cases with CCN syndrom have shown that the determination of thiamine content in tissues and erythrocyte transketolase activity is the most important from the point of view of diagnostics and differential diagnostics. In experimentally induced CCN syndrome by the Amprolium administration the most significant decrease of thiamine content was found in brain tissue, the value falling by 75 % in average. A pronounced decrease of thiamine content was observed also in muscles. The lowered thiamine content in liver of calves following the administration of Amprolium was found to be insignificant. In contrast to these findings a highly significant fall of thiamine content in liver occurs in spontaneous CCN in calves (Edwin et al. 1979; Jagoš et al. 1981).

The values of TPP effect are indirectly indicative of the saturation of organism with thiamine. Already 14 days after the administration of Amprolium the TK activity decreased while the TPP effect was found to increase in calves with the experimentally induced CCN. The increase of the TPP effect observed by us was always substantially lower than the values reported by Edwin et al. (1979). When studying the dynamics of plasma levels of vitamin A, E and C, of minerals Na, K, Ca, Mg, inorganic P, Zn, and Cu, of urea, glucose, aspartate aminotransferase and gamma glutamyl transferase activity, blood acid-base balance and of some other parameters in rumen fluid no significant differences were found between the ex-

perimental and control group. Normal serum levels of Ca, Mg, Na and K in Amprolium-treated calves stated also Lilja (1973) whereas Markson et al. (1966) found decreased plasma levels of potassium.

It follows from our studies of cerebrocortical necrosis and of experimentally induced CCN syndrom that for establishing an objective diagnosis of CCN the knowledge of reliable history together with a repeated clinical examination are essential. The pathologico-morphological findings in the cerebral cortex (Terlecki, Markson 1961; Pivník 1972) and the determination of the lower thiamine content in liver, brain and muscle tissue (Jagoš et al. 1981) have the decisive significance for the confirmation of diagnosis as well as for the differential diagnosis. The preclinical stage of CCN could be diagnosed on the basis of the incidence of lower TK activity in blood and of increased TPP effect. The improvement in the condition of affected calves following the administration of thiamine could be used for indirect diagnosis.

It follows from the results obtained by the studies of symptoms in Amprolium-treated calves that the development of the experimentally induced disease somewhat differs from the spontaneously occurring CCN syndrome. It can be concluded that the CCN syndrome experimentally induced by the administration of Amprolium is not a suitable model for the study of the pathogenesis and symptomatology of spontaneously developed CCN in calves.

Syndrom cerebrokortikální nekrózy experimentálně vyvolaný aplikací amprolia u telat

V experimentu, na 3 pokusných a 4 kontrolních telatech byl aplikací Amprolia vyvolán syndrom cerebrokortikální nekrózy (CCN). Byla sledována patogenéza a symptomatologie vyvolané poruchy. Klinické příznaky se objevily u 2 telat za 6 týdnů po intraruminální aplikaci Amprolia (0,5 g/tele/den), u třetího telete za 54 dnů. Hlavními klinickými příznaky byly inapetence, slepota, zvýšená dráždivost, příznaky statické a kinetické ataxie. Telata s obtížemi vstávala, narážela do překážek, objevovaly se svalové třesy, opistotonus, a při upadnutí tonicko-klonické křeče a plovací pohyby. Zjištěné klinické příznaky byly totožné s projevy spontánního onemocnění telat CCN.

Синдром церебро-кортикального некроза, экспериментально вызванный применением ампролия у телят

В эксперименте над 3 подопытными и 4 контрольными телятами был вызван применением ампролия синдром цереброкортикального некроза (СЦН). Проводились исследования патогенеза и симптоматологии вызванного нарушения. Клинические признаки появились у 2 телят через 6 недель после внутриручного введения ампролия (0,5 г/теленка/сутки), у третьего теленка — через 54 дня. Главные клинические признаки — отсутствие аппетита, слепота, повышенная раздражительность, признаки статической и кинетической атаксии. Телята с трудом поднимались, негалкивались на препятствия, появились мышечные дрожания, опистотонус и при падении тоническо-клонические судороги и плавательные движения. Выявленные клинические признаки были схожи с проявлениями спонтанного заболевания телят СЦН.

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