Some publications about the BSE (2001- 2003) and other supporting literature sources

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Introduction

Recently Italy was hosting the third annual edition of NeuroPrion (October 4, 2006), the leading international forum on mad cow and similar new diseases caused by mysterious bodies called prions. This third mad cow forum brings together **more than 1,000 experts from 52 research institutes in some 20 countries.** The conference examined possible new variants of mad cow and the risk of other prion diseases crossing the species divide from animal to man…The **mad cow crisis cost Europe more than 90 billion euros**…(http://www.italymag.co.uk/2006/news-from-italy/current-affairs/italy-hosts-third-mad-cow-forum/). However, where are (Internet) conclusions about „**the BSE infectiosity as the result of this forum“?**

Ten years ago, a new strain of human brain disease was identified, and it sparked one of the great medical scares of modern times. New variant Creuzfeldt Jakob Disease was not only a degenerative and incurable infection of the brain, it was linked to the outbreak, a decade earlier, of a similar disease in cattle - BSE. In 1996, after months of foot-dragging, John Major's government was finally forced to announce that vCJD could be caused by eating contaminated meat. The statement led to the virtual collapse of the **British beef industry, an export ban, a cost of some £3.5 billion** and - since thousands of people in Britain had been exposed to the risk by eating infected beef - the possibility of a serious pandemic. Newspaper headlines spoke of hundreds of thousands of deaths from this deadly disease. Even scientists analysing likely models for the outbreak admitted that up to 80,000 people could die. However, the yearly figure has been in steady decline since 2000 - when 28 people died. **Whatever happened to the great epidemic?**

The headquarters of the surveillance unit is an anonymous office block at Edinburgh's Western General Hospital and is run by Dr Richard Knight, a 55-year-old clinical neurologist. His job is to monitor the progress of the disease, to plot how or whether it is changing and to inform scientists, and ultimately the public, if we are at risk. He is not only responsible for the UK's state of knowledge on the various strains of CJD, he also co-ordinates research across Europe. **Why are the figures falling?** With refreshing candour, he admits, "We do not know." But the more he describes the complexities of the disease, the accepted scientific definition of its nature and the dominant theory that links it to cattle, the more I begin to question whether we might not have got the whole thing badly wrong, and to wonder whether

there really is any connection between the cattle and the human disease. **Could this have been the scare that never was?** (LINKLATER, 2006).

HLÁSNÝ,J.: Nervous diseases and connections with nutrition in ruminants. Výzkum v chovu skotu (Rapotín), 43, 2001 (1); 13-29

Causes of the tendency to hypomagnesemia and hyperammonemia

Over the past 50 years yields of many crops have increased roughly in proportion to the increase in NPK–fertilizer application. Luxury consumption of K fertilizers, leads to distortion of cation ratios in the herbage: concentration of Na, Mg and Ca are reduced relative to potassium. Higher N–fertilization increases N concentration, and appears to increase the concentrations of P and K in feedstuffs, and decreases Mg utilization by livestock with the occurence of hypomagnesemia. Also, if the P content in young pasture is high, precipitation of insoluble guanite ($MgNH_4PO_4$. $6H_2O$) may begin in the rumen which decreases Mg utilization. Higher N concentration in forages gives higher contents of higher fatty acids and decreases the availability of feed Ca and Mg by the formation of insoluble Ca and Mg soaps in the gastrointestinal tract. Highly fertilized young herbage is characterized by a high content of crude protein (CP) and a high rate and extent of degradation of CP causing high concentrations of ammonia–N in the rumen. At elevated blood concentrations ammonia is toxic to the central nervous system.

Elimination of excess ammonia and the „magnesium control" of ornithine cycle in liver

In the ornithine cycle, urea synthesis is generally considered as a mechanism for the elimination of excess ammonia. The urea level in blood of animals is closely related to rhythmic intake of dietary protein and is strictly reversely proportional to the biological value of the dietary protein. Also, the high ratio of protein to energy increases the urea levels in blood. The sum of the two processes, amino acid catabolism and urea synthesis does not result in accumulation of NH_4 cation in the blood. However, a high rate and extent of degradation of CP causing high concentrations of ammonia–N in the rumen results in hyperammonemia, because of diminished capacity of the liver to synthetise urea, and to a decrease in glutamine synthetase.

In the fed state, most amino acids reaching the liver, except for the branched-chain amino acids, can serve as precursors for ureagenesis in ornithine cycle. Control of this metabolic pathways can either be long term involving the synthesis and degradation of enzyme molecules (time ranges of hours or days), or short time, via activation or inhibition of existing enzyme molecules (time range of seconds or minutes). So, increases in enzyme concentrations are observed after feeding a protein rich diet. Together with the short-term control mechanisms of ornithine cycle activity these long-term adaptations allow a large changes in flux through the ornitine cycle at a relatively constant ammonia concentration. Of prime importance in the short– term control of carbamoyl– phosphate synthese activity is the mitochondrial concentration of N–acetylglutamate, a compound that is indispensable for enzyme activity.

However, the concentration of mitochondrial free Mg^{2+} may be relevant, since binding N-acetylglutamate to carbamoyl– phosphate synthase is dependent on this action. The Ca^{2+} can compete with Mg^{2+}, an increase in the extramitochondrial free Ca^{2+} concentrations in the physiological range (from 0.1 to 0.7 uM/l), stimulates citrulline synthesis, whereas higher Ca^{2+} concentrations are inhibitory.

Hepatic encephalpathy and a some exceptions in ruminants

Also, chronic and acute liver insuficiencies are associated with increased blood ammonia levels. Although there is strong evidence that ammonia is a major neurotoxin, its mechanism of action is still a matter of debate, because the clinical symtoms in chronic hepatic encephalopathy (HE) are reversible. However, the terminal phase of acute HE, as is observed in fulminant hepatitis, is irreversible and is associated with brain edema.

The concentrations at which ammonia becomes neurotoxic appear to be rather variable. In encephalopathy resulting from acute ammonia intoxication, the letargy occurs when brain ammonia concentration increases to 750 uM/kg. Ammonia has major effects both on excitatory and inhibitory synaptic transmission. It was suggested that in the early stage of acute HE, the inhibitory postsynaptic potential (IPSP) is decreased and that both IPSP and excitatory postsynaptic potential (EPSP) are decreased in later stages.

The basic process involved in chemical synaptic nerve transmission is an electrical signal in the presynaptic terminal tranduced into the secretion of a chemical signal that serves as the message to the postsynaptic cell. Only two types of synaptic potentials were recorded: (1) activation of excitatory pathways evoked excitatory postsynaptic potentials (EPSP) in which a very brief (at most a few miliseconds) increase in conductance to cations depolarized the membrane. (2) activation of inhibitory pathways evoked inhibitory postsynaptic potentials (IPSP) in which also a brief increase in ionic conductance, primarily to chloride ions, usually resulted in a hyperpolarization. These synaptic potentials are mediated by amino acids: glutamate and aspartate for EPSP and gama– aminobutyric acid and glycine for IPSP.

Tetany is the classical symptom of Mg deficiency. The biological bases of tetany are vary complex because it involves: the central nervous system, the peripheral nervous system, the neuromuscular junction and the muscle cell itself. The cerebrospinal fluid and the electroencephalogram are generally normal in Mg-deficient animals, but these animals are more sensitive to tetany– inducing stimuli such as sound or electroshock. Audiogenic seisure susceptibility occurs in rats if cerebrospinal fluid Mg concentration is low, but the cerebrospinal fluid Mg is often normal in Mg deficient tetanic animals. However, a decrease in concentration of Mg in cerebrospinal fluid (CSF) was occurred in sheep and in cattle – with the clinical signs of hypomagnesaemic tetany correlated with a CSF Mg decrease. In both species the Mg concentration of lumbar CSF decreased from a normal level of about 0.9 mmol/l to about 0.5 mmol/l by the time tetany supervented. It seems that the Ca pump of the sarcoplasmatic reticulum is pertubed since it is strongly Mg dependent. Thus the leakage of Ca from the sarcoplasmatic reticulum pump, inducing the contraction of the muscle fibrils, seems to be the most probable mechanism responsible for tetanic seisures of Mg deficiency.

Magnesium and calcium antagonism in the neurotoxicity

In the central nervous system (CNS) magnesium (Mg^{2+}) ion has two major functions: the stabilization of synaptic connectivity and widespread enhancement of neurochemical enzymatic functions. The Mg^{2+} has been shown to affect guanine nucleotide binding proteins (G proteins) in several ways: nanomolar concentrations of Mg^{2+} are required for GPT-ase activity, micromolar concentrations of Mg^{2+} are required for receptor mediated activation of G proteins, milimolar concentrations of Mg^{2+} increase the affinity of various types of receptors for agonists, an effect thought to result from increased receptor– G– protein coupling, voltage– dependent– Ca^{2+} channel, and N–methyl – D – aspartate (NMDA) receptor operated ionic channel.

Because Ca ions are normally low in most body cells (less than 10^{-8} M), compared with the ECF Ca level (2 mM), only a small amount is needs enter to significantly increase ICF Ca level. Neuronal free calcium concentrations correlates with the likelihood of irreversible ischaemic cell death, and free intracellular Ca increases may result from Ca entry via the

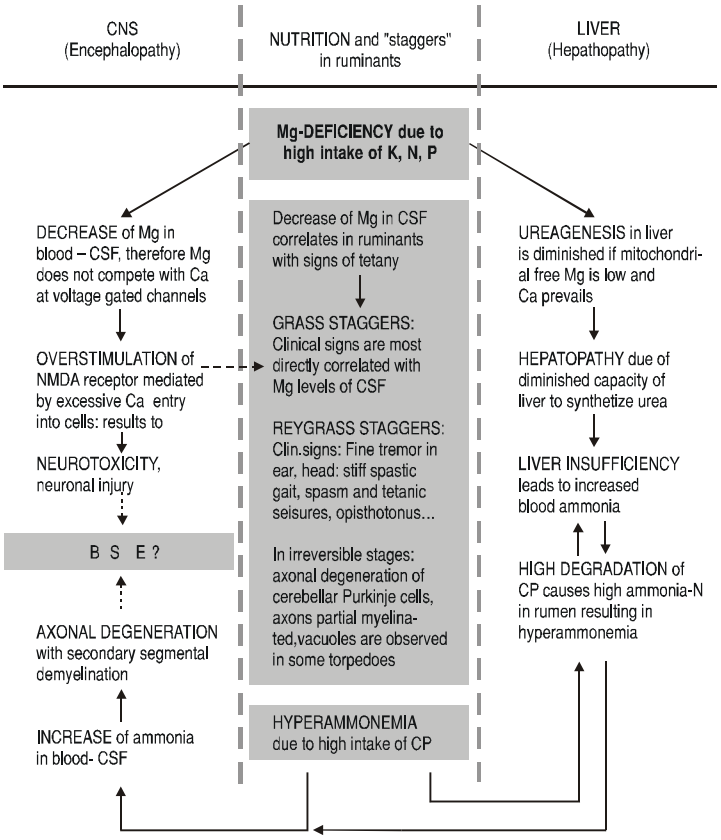
NMDA ion channel and voltage – gated calcium channels, and release from endoplasmic reticulum and other intracellular stores. Magnesium competes with calcium at voltage– gated calcium channels both intracellularly and on the cell surface membrane. It may thereby impede Ca– dependent presynaptic release of glutamate and prevent neuronal Ca overload via voltage– gated channels during ischaemia. Magnesium also enhances mitochondrial buffering of raised intracellular free calcium ions, and prevents release of intracellular calcium stores from endoplasmic reticulum.

Magnesium and ammonia antagonism in neurodegeneration

Under normal or moderate hyperammonemic conditions, flux through glutamate dehydrogenase is in direction of ammonia formation. Results on the effect of ammonia on glutamate and aspartate levels in brain are numerous. Both acute and chronic hyperammonemia are associated with a decreased brain asparate concentration. Excitatory amino acids such as glutamate and aspartate are major neurotransmitters in the mammalian CNS. It is generally accepted that these aminoacids are primarilly responsible for normal excitatory synaptic transmission. These neurotransmitters produce their effects by interacting with specific receptors on the cell surface, the excitatory amino acid receptors. Five receptor subtypes have been identified, however the most well characterized excitatory amino acid receptor is NMDA receptor which also is permeable to Ca^{2+}. Overstimulation of the NMDA receptor as well as other excitatory amino acid receptors results in neurotoxicity and neuronal injury. These receptors are considered as the final common pathway for many acute and chronic neurologic conditions. An important consequence of NMDA receptor activation is the influx of Ca^{2+} into neurons. Excessive NMDA receptor stimulation is thought to be an important factor in neuronal cell damage, mediated by excessive calcium entry into the cell.

Fig. 1

Nervous diseases and connections with nutrition in ruminants



CNS: Central nervous system • CSF: Cerebrospinal fluid • CP: Crude protein
NMDA: "N-Methyl-D-Aspartate" receptor.

Studies have demonstrated that magnesium can protect against NMDA– induced neurodegeneration, brain injury, and convulsions. On the other hand, hyperammonemia it appears to be a cause of primary axonal degeneration with secondary segmental demyelination: the segmental loss of myelin is consequent to an abnormality in the axon cylinder, which probably reflects a metabolic failure of the perikarion...

Grass staggers, neurological signs and neurodegeneration

According to the literature, mostly staggers are caused by ingestion of forages that contain tremorgenic mycotoxins. The mechanism of action of these toxins is often incompletly und*e*rstood, but may involve enhanced release of excitatory amino acid neurotransmitters. However, forages causing staggers in livestock include perennial ryegrass must be considered in the differen*t*ial diagnosis of hypomagnesemia. Expression of clinical signs is most directly correlated with CSF Mg levels (less than 1.45 mg/dl). However, it is possible to have normal plasma Mg levels, particularly if violent convulsions have been occuring. Commonly, grass staggers (grass tetany) usually occurs in lactating cows during winter or spring when grasses are low in Mg, especially in cattle experiencing less than optimum energy intake, and during an adverse weather. Affected cows, also may be pastured on rapidly growing planted forage crops such as wheat, barley,or oats that have been over fertilized with nitrogen and potassium.

In reygrass staggers animals appear normal at rest or may have fine tremor in the ear and head. When stimulated, affected animals have a characteristic stiff, spastic gait, followed by spasmus and tetanic seisures, opisthotonus occurs in severe cases. Recovery from an episode may be rapid for perennial ryegrass, dallisgrass, and bermudagrass staggers if animals are not stressed. Seisure episodes dissipate within 2 weeks after removal from the toxic forage.

However, in irreversible stages (longer pasture standing) axonal degeneration of cerebellar Purkinje cells has been found. These lesions consist of eosinophilic homogenous swellings in the cerebellar layer, generally located in groups rather than randomly distributed and with the tendency to be more numerous adjacent to the Purkinje cell layer. The axons were myelinated at least over part of their traceable length, often on both sides of the swelling. The myelin sheats would appear to remain relatively unaffected about degenerating axons, vacuoles were observed in some torpedoes. Similar lesions in man can be found include subacute cortical atrophy and multiple- sclerosis. It appears that the longer the disturbing syndromes has been present, the greater the likelihood of finding these axonal changes in cattle and sheep.

Generally, it seems that diseases of the nervous system in ruminants are in some connections with the nitrogen– magnesium metabolism. The surpluss of nitrogen and potassium intake can have association with hyperammonemia complicated with subclinical hypomagnesiemia, and the neurodegeneration may be involved. These mechanisms should also be studied in bovine spongiform encephalopathy (BSE) which can be on these mechanisms based. The epidemiological studies show, that BSE occurence to this date is only in western european countries where significantly higher NPK fertilization is applied. This review is based on the author´s experiences from WVU (1991) where hypomagnesemia and high BUN levels were found in connection with high nitrogen and potassium intake in grazing ewes.

HLÁSNÝ,J.: Nervous diseases and connections with nutrition in ruminants, or only the story of BSE yet? Výzkum v chovu skotu (Rapotín), 44, 2002 (4): 10-33

Bovine spongiform encephalopathy (BSE) belongs to the family of diseases known as the

transmissible spongiform encephalopathies (TSE´s). These diseases are caused by similar uncharacterized agents that produce spongiform changes in brain. In the next decades, a series of experiments , many led by professor Prusiner, demonstrated that prion protein (PrP) actually is present in healthy animals, but in a different form from the one found in diseased animals. While many of Prusiner colleagues have come to accept the once heretical prion theory, most say it still faces some crucial unanswered questions. Also an important remained question is; where are used exports of „infectious" Britain meat and bone meal (MBM) in cattle feed? Eurostat data (1990-2000) indicate that UK exports of MBM averaged 20,000 tonnes in 1990- 1996 (totalling 138,000 tonnes over the period) and 23,000 tonnes in the 1997- 2000 period(91,000 tonnes in total). Where these exports used in cattle feed? And to where were they shipped? Nobody knows… (ZIGGERS, 2001). However, meat products are more valuable for simple- stomached than for ruminant animals, since the latter do not require a dietary supply of high quality protein. Both meat meal and MBM are eaten readily by pigs and poultry, and may be given at levels of up to 150 kg/t of the diet for laying hens and young pigs…, these products are not readily acceptable to ruminants , and must be introduced into their diets gradually. Meat meal generally contains from 600 to 700 g/kg of protein compared with about 450 to 550 g/ kg for MBM. Therefore, considerable care is required in storing the meat products to prevent the development of rancidity… (McDONALD et al., 1988).

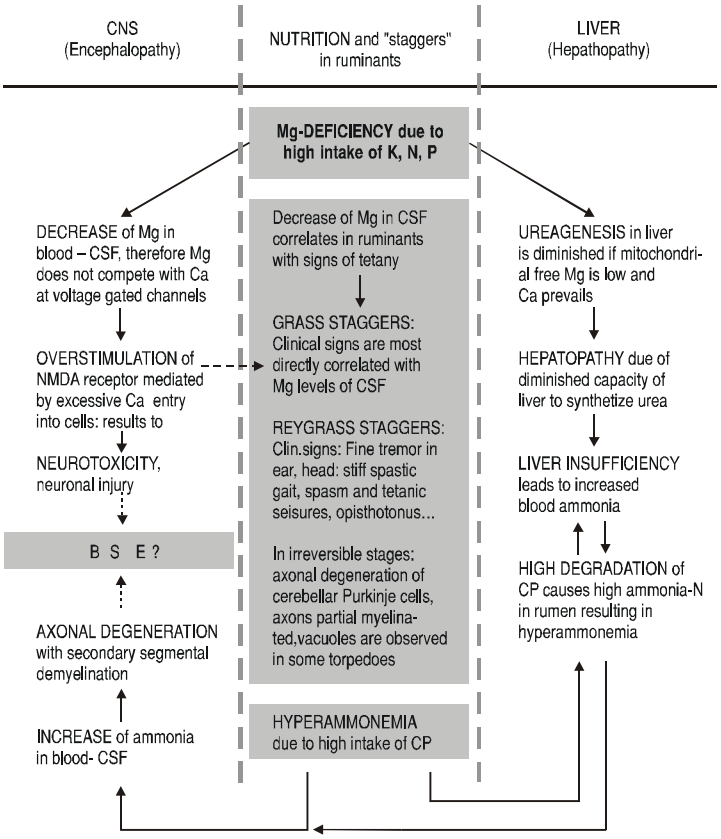
However, recent report from MOORBY et al.(2000) describes a nutritional experiment in forty- seven multiparous Holstein-Friesian dairy cows, as a different cause of BSE. During the last six weeks of the dry period they were offered one of three grass silage (first cut perennial ryegrass)- based diets, offered ad libitum. After calving, all the animals received the same lactation diet consisting of ad libitum access to ryegrass silage... After the 21 weeks of lactation, six from the 47 animals developed clinical signs of BSE, which was later confirmed by histopathological examination. The differences observed indicate that the energy metabolism of dairy cows incubating BSE may be subtly altered before the onset of clinical signs of the disease (MOORBY et al., 2000). In addition the BSE developed after 27 weeks of ryegrass feeding- without meat and bone meal in the experimental diet. Unfortunately, without the Mg status of cows testing.

Therefore, the feeding of MBM to cattle per se may not be the main cause of BSE. However, by excessive feeding of MBM, especially in dairy cows, a state of hyperammonemia can be achieved and hypomagnesemia can also be initiated. When this article about „ryegrass toxicity" on the CNS of cows was published; it was prompted to air my views in studies of CNS neurotoxities by nutritional causes. I reviewed about 200 papers on the CNS changes associated with BSE, and detected a possibility that these mechanisms have a strong influence on CNS, especially in ruminants, and that the BSE has its roots in a more common nutritional problem (HLÁSNÝ, 2001). It seems that this alternative „Czech ammonia- magnesium theory"(based on the chronic Mg-deficiency potentiated by hyperammonemia in ruminants) versus other infectious theories can be useful to change the „official" view on the BSE, and the vCJD research.

This theory was introduced with the well- known fact, that over the past 50 years yields of many crops have increased roughly in proportion to the increase in NPK–fertilizer application. Luxury consumption of K fertilizers, leads to distortion of cation ratios in the herbage: concentration of Na, Mg and Ca are reduced relative to potassium. Higher N–fertilization increases N concentration, and appears to increase the concentrations of P and K in feedstuffs, and decreases Mg utilization by livestock with the occurence of hypomagnesemia. Also, if the P content in young pasture is high, precipitation of insoluble guanite ($MgNH_4PO_4 . 6H_2O$) may begin in the rumen which decreases Mg utilization. Higher N concentration in forages gives higher contents of higher fatty acids and decreases the availability of feed Ca and Mg by the formation of insoluble Ca and Mg soaps in the

gastrointestinal tract. Highly fertilized young herbage is characterized by a high content of crude protein (CP) and a high rate and extent of degradation of CP causing high concentrations of ammonia–N in the rumen. At elevated blood concentrations ammonia is toxic to the central nervous system. It was described in the bulletin of Research Institute for Cattle Breeding (Rapotín) as the following figure (HLÁSNÝ, 2001; see also „Feed Mix“, vol.10, No 2, 2002:12-15, http://www.agriworld.nl/feedmix/headlines.asp?issue=3) :

Obr. 1 Nervous diseases and connections with nutrition in ruminants



CNS: Central nervous system • CSF: Cerebrospinal fluid • CP: Crude protein
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In the figure imagined nervous diseases in ruminants; there is the main idea to show the hypomagnesemia plus hyperammonemia „simultaneous“ action on the animal tissues (especially tissue of CNS and liver). If the BSE is involved; a long- chronic action of biochemical changes in the blood- CSF is necessary to rise irreversible neurodegenerative changes. As a typical example; the ryegrass staggers was showed. The various clinical symptoms can be observed because the nervous system controlling both voluntary and unvoluntary muscles is affected. Also variations in the frequency with which some signs can be recorded in animals observed at different times during the nervous disease. It seems, that during the chronic hypomagnesemic disease, the heavy weather (cold- rainy, windy...) or nutrition (hyperammonemia...) stress - the episodes of acute abruptions, may accelerate the nervous „BSE“ disease.

Personal experiencies with the NPK-fertilization, and the hypomagnesemia in ruminants

I participated on the grazing trial at the West Virginia University (COX GANSER et al. 1992); there the metabolism of 24 ewes has been tested at three different pastures during the

five weeks period. Pastures were fertilized with 90 kg N/ ha (as N-nitrate) in late March. Ewes were fed a good quality grass legume hay (CP- 11%, K- 1.9% of dry matter) after lambing in late February , and in mid April six groups of six ewes (3 with twin lambs, and 3 dry) were allocated to three pastures (Matua, Tall fescue, Kentucky bluegrass with red clover). Pasture samples were taken for mineral analysis (N, P, K, Ca, Mg, S, Mn, Fe, Cu, B, Al, Na), and also samples of the blood (glucose, urea, cholesterol, total protein, creatinine, albumin, globulin, bilirubin, triglycerides, ALP, LDH, SGOT, SGPT, GGT, amylase), and urine samples (Ca, P, Na, K, Mg) were taken. After five days of young pasture in lactating ewes feeding (CP; 20-30%, K; 2.4- 4.0% of dry matter):

- Mg blood values significantly decreased(from 1.7 to 1.1 mg/ dl)
- with previously significantly increased blood urea (from 20 to 45-50 mg/dl)
- with following significant AP increaee (from 100 to 200-340 IU/l, and with a slightly increase of AST (from 140 to 160- 340 IU/l)).

In addition, blood and urine concentrations of Mg fell further to the end of experiment (matua pasture, especially), and did not recover to the same extent as it was at the begining of pasture feeding. This experiment shows, that spring pasture feeding , it is an example of the nutrition changes (especially in hypomagnesemia, and uremia), after the winter feeding, when N and K levels in feeeds are low compared with their level in young pasture.

Concerning the human nutrition, the high NPK fertilization have some connections with the cow´s milk mineral composition, especially about the decrease of the Mg content (HLÁSNÝ and STEIDL, 1990).

What is the foundation of a long „dangerous“ incubation period in BSE?

The BSE was initially recognized in cattle in the UK in 1986; there is good information that it had not occurred before then. There is no evidence that BSE spreads horizontally, i.e., by contact between unrelated adult cattle or from cattle to other species. Epidemiological research led to the conclusion that the bovine agent had originated from the scrapie agent, which had been present in sheep in the United Kingdom for at last 200 years. It is presumed, but will likely never be proven, that the scrapie agent jumped species and moved into cattle when sheep offal was included in protein supplements fed to cattle. After cattle started to die, cattle carcasses and offal were included in the same protein supplements. However, after ban on ruminant protein (meat and bone meal) in ruminant feed (July, 1988), and the following significant decrease of BSE incidence in the UK (1994)- this seems to have amplified the „BSE story“. This was a reason for foundation idea of a long incubation period of BSE (five years or more…).

Why the BSE began in the United Kingdom ?

a/ According to the scientific „official“ theory:

The BSE was initially recognized in cattle in the UK in 1986; there is good information that it had not occurred before then. The theory favoured by most scientists who have studied the disease is that it originated from an infection by scrapie in sheep. It began in the UK and not elsewhere because of a comparatively high incidence of scrapie in UK sheep and a comparatively large proportion of sheep in the mix of carcases rendered for animal feed for livestock.

b/ According to the Czech „ammonia- magnesium“ theory:

It began in the United Kingdom (UK) because of there the „ideal“ rainfall, and the available water capacity of the soil for high NPK fertilizers utilization by grassland (mostly ryegrass). This is a major determinant for high grasses yield ranging in the UK from 6000-14000 kg DM/ha under intensive fertilization. However, there are notable exceptions such as

Benelux , which although characterized by the highest pasture yields in Europe, has a comparatively low share of grassland in total ruminant feed composition (50- 55%) – compared with Ireland-97%, UK- 83%, France- 71%. On the other hand, the permanent grazings of the Mediterranean zone are subject to severe moisture stress with annual production being limited to about 1000 kg DM/ ha. However, in this climatic zone, irrigated legume and legume/ grass swards are capable of outputs of 20000 kg DM/ ha (LEE, 1988)- for example see Portugal (mostly marine climatic zone), and high incidence of BSE …

The high consumption of nitrogen, and potassium fertilizers in the UK (continued during 1985- 1990 period) yearly averaged around 1.6 and 0.5 million, with a change in 1995 to 1.4 and 0.5 million tons, resp. (93 and 100%, resp.). For example in Czechoslovakia, there a significant „ecological" changes were observed in this period -decrease of N and K-fertilizers consumption to 47 and 12%, resp.(HLÁSNÝ, 1997). Therefore, together (in Britain); marine climatic zone, high concentration of cattle and sheeps (high production of animal excrements), there are ideal conditions for intensive grassland fertilization.

In Britain perennial ryegrass is the most important species of sown pastures. The composition of the dry matter (DM) of pasture is very variable: for example, the crude protein (CP) may range from as little as 30 g/ kg in very mature herbage to over 300 g/kg in young, heavily- fertilized grass. The digestibility of the organic matter is one of the main factors determining the nutritive value of forage, and this may be as high as 0.85 in young spring pasture. The digestibility decreases as plants mature, the relationship is complicated by there being a spring period of up to a month during which the herbage digestibility remains fairly constant – as a „plateau"(McDONALD et al., 1988). Therefore, in Britain, grasses contain relatively high CP content (more than 25%),and lower Mg content (less than 0.2%). However, these conditions are the cause of hyperammonemia and hypomagnesemia - an inadequate level of magnesium (Mg) in the blood. It most commonly occurs among lactating animals grazing rapidly growing, lush spring pastures containing less than 0.2 % Mg and more than 25% of crude protein (ENSMINGER et al., 1990).

Why the BSE began in the mid- 1980s?

a/ According to the scientific „official" theory:

Because of the elimination several years earlier of a step in tallow extraction from rendered carcases that allowed some tissue infected with scrapie to survive the process and to be recycled as cattle adapted scrapie or BSE.

The opposite opinion to this theory:

However, experiments to test the point with brain tissue infected with scrapie or BSE showed that the inactivation produced by the tallow extraction step (organic solvents and steam) was not very impressive – on average only about 10 median lethal doses (10 log LD_{50}) per mililitre. Nevertheless, if infectivity was present at a concentration of less than 10 log LD_{50} / ml before tallow extraction, which seems highly probable, then the elimination of a step that had caused a one log reduction might well have been sufficient for infectivity to survive the process and contaminate the resulting meat and bone meal feed. The probability of an input of infectivity considerably lower than 10 LD_{50}/g in the carcases coming to a rendering plant can be appreciated by some simple arithmetic. The weight ratio of carcases to processed meat and bone meal is around 5 to 1. Thus, an input infectivity of 10 LD_{50}/g would be concentrated into 0.2 g of meat and bone meal. A growing calf consumes about 2 kg of feed daily, of which meat and bone meal constitues 4.5% by weight, or 90 g meat and bone meal., containing 4500 LD_{50}. Because 1 LD_{50} is defined as the amount of infectivity with a 50% probability of killing an animal, and even taking into consideration the effects of the different species and route of infection in natural versus experimental BSE, it could be

reasonably surmised that a daily intake of 4500 mouse intracerebral LD_{50} would have very likely killed every calf in the UK years ago (BROWN, 2001).

b/ According to the Czech „ammonia- magnesium“ theory:

Because in the mid- 1980s (see the NRC; 1984, and 1988), it is a begining of the higher dietary protein (16.6- 16.8%) recommendation including undegradable protein (meat and bone meal..) incorporation, especially to lactating cow rations. This helps to stimulate feed intake and permits efficient use of mobilized body tissue- for higher and higher milk production. So, high producing cows generally require additinal nondegraded protein sources beyond their normal ration to meet total protein requirements: the higher milk production, the greater the quantities of undegradable protein (UIP) needed. However, the NRC recommendations were „overdosed“ in the dairy praxis to 19% or more crude protein to meet the requirements during early lactation, with only 0,2% of the Mg (ENSMINGER et al., 1990). The same high protein recommendations (18.8%) are from McCULLOUGH (1994) for high producing dairy cows, however with higher (0.33%) magnesium content in dairy rations.

Approximately 60% of the crude protein in the typical dairy cow ration is broken down – degraded (DIP) by microbial digestion to ammonia (NH_3). The rumen microbes must convert the NH_3 to microbial protein in their own cells if the dairy animal is to receive any benefit. Fermentable energy must be available for the organisms to grow and synthesize the necessary amino acids. If rumen NH_3 levels are excessively high, the NH_3 is absorbed into the blood and either recycled or excreted in the urine as urea (ENSMINGER et al., 1990). Excess NH_3 in the portal system can readily pass through the liver and enter the arterial system. Ammonium ion (NH_4) in the bloodstream is in equilibrium with NH_3, which can cross the blood- brain barrier at a rate dependent on the speed of dissociation of NH_3 from NH_4 at the level of the cell membrane. Brain tissue rapidly extracts NH_3 from the arterial blood. Within the critical blood concentration range, it appears that the animal cannot detoxify ammonia fast enough to keep ahead of absorption from the stomach (rumen). In other words, the urea and glutamine- synthesizing mechanisms are saturated. Ammonia then builds up in bloodstream (hyperammonemia) and more and more NH_3 accumulates in tissue cells. The CNS is first to malfunction because it has a large requirement for energy. Behavioral and nervous signs do seem to appear first, cellular energy and respiration deficits probably cause ultrastructural damage and the degenerative changes (BOOTH and McDONALD, 1988).

Are common the clinical signs of BSE and magnesium deficiency in cows?

a/ The clinical signs of BSE:

Most cases of BSE in Great Britain have occurred in dairy cows between 3 and 6 years of age with the initial clinical sigs : nervous, kicking, locomotor difficulty, loss of condition, loss of weight, reduced milk yield, abnormal behaviour, nervous of entrances, temperament change, falling, aprehension, aggression, dificulty rising, tremors, hyperaesthesia, and recumbency. Other clinical signs analysed for the 17,154 cases; ataxia, kicking in parlour, excessive licking, head pressing or rubbing, abnormal ear position, teeth grinding, abnormal head carriage, head shyness… There were variations in the frequency with which some signs were recorded in animals observed at different times during the epidemic (WILESMITH et al., 1992).

b/ The causes and clinical signs of hypomagnesemia

Forages causing staggers in livestock include perennial ryegrass must be considered in the differential diagnosis of hypomagnesemia. Expression of clinical signs in „ryegrass staggers „ is most directly correlated with the cerebrospinal fluid (CSF) Mg levels (SMITH, 1996). Several factors adversely influence Mg metabolism in cattle and may „trigger“ grass tetany.:

among them drastic fluctuations in spring temperatures, prolonged cloudy weather, organic acid content of plants, hormonal status of the animal, level of higher fatty acids in plants, energy intake of the animal, and additional stress – such as a dog chasing animals, parasites, or a cold rain. Grass tetany is most likely to occur on pasture plants grown on soils that are low in available Mg and high in available potassium. When the ratio K/(Ca+Mg)in the feed ration is higher than 2.2, it is likely to induce hypomagnesemia (KEMP and t´HART, 1957). Therefore, if calcium is low as well as Mg, the hazard of tetany is even greater. However, in the UK there is well known that rainfall can affect the mineral composition of pasture herbage. Calcium, for example tends to accumulate in plants during periods of drought but to be present in smaller concentration when the soil moisture is high. There the exact cause of hypomagnesaemic tetany in ruminants is unknown, although a dietary deficiency of Mg may be a contributory factor. In addition, in britain book „Animal Nutrition", there are no informations about the potassium content in forages (McDONALD et al., 1988). Also, according to recently well known book (SMITH, 1996), there is only little informations about cow hypomagnesemia in the UK. There, from more than 40 references, only two are about Mg- research in the UK (MOODIE,1965: COLLINS,1980), signalize however, that there hypomagnesemia- hypocalcemia, can would be a problem.

A deficiency of Mg may cause grass tetany (grass staggers) in cattle; lactating ewes and dairy goats are also susceptible. It is highly fatal, affecting only ruminant species. Hypomagnesemia is usually accompanied by hypocalcemia. The highest incidence is in high-producing cows in the third to fifth lactation, within 60 days of calving that are pastured on cool season grasses. Generally, occurs within the first 2 weeks after animals are turned out on new pasture growth, either in spring or fall. Also, the disease is almost likely to strike beef cows during early lactation, especially those with high levels of milk production. Dry cows and bulls are seldom affected. Older cows are more susceptible to grass tetany than those with their first or second calves, because of lowered Mg stores; decreased absorption efficiency, and reduced ability to resorb adequate amounts of Mg from the bone.

The initial signs include nervousness, attentive ears, markedly erect ears, ear twitching , hyperestesia, and decreased milk yield are early clinical signs. Cows are alert and hyperexcitable, and they may charge. In more severe cases affected animals may avoid the rest of the herd, walk with a stiff gait, lose their appetite, and urinate frequently. They are nervous, have staring eyes, keep their head and ears in an erect position; twitching of muscles (usually of head and neck), head held high, accelerated respiration, higher temperature, grinding of the teeth, and abundant salivation. Also, they stagger: have a twitching skin, especially on the face, ears, and flanks: and lie down and get up frequently. Animals may be irritable and behave aggressively : they may even charge or fight persons in the immediate area. After a time, extreme excitement and violent convulsions may develop. Animals lie flat on their sides, the fore legs pedal periodically, saliva flows freely, breathing is labored, and the heart pounds rapidly. Violent episodes of opisthotonus and clonic convulsions can be precipitated by any stimuli, and these alternate with periods of tetanic muscle spasms. Nystagmus, exaggerated mastication, and a snapping eyelid retraction occurs. If treatment is not given at this stage, animals usually die during or after a convulsion (SMITH, 1996).

The various symptoms of animals suffering from grass tetany indicate that the nervous system controlling both voluntary and unvoluntary muscles is affected. The Mg concentration must be maintened for the normal production and decomposition of acetylcholine. Low Mg: calcium ratio potentiate acetylcholine release, and alterations of the ratio in the extracellular fluid may contribute to tetany. If uncontrolled, the tetany spasms culminate in cardiorespiratory failure (ENSMINGER et al., 1990; SMITH, 1996). However, according to professor Bečka findings, not only Ca – Mg disturbances, but also acidemia (surpluss of H ions) cause the inhibition of the sympathetic, and the parasympathetic nervous system action

prevails (HLÁSNÝ, 2000; HLÁSNÝ, 2000a; HLÁSNÝ, 1999).

Because the tetany can develop within a day or two of animals being turned out to graze, the condition has been referred to as the acute form. In the chronic form of the disease plasma magnesium levels fall over a period of time to low concentrations. This type is not uncommon in suckler herds. Clinical signs of the disease are often brought on by „stress" factors such as cold, wet and windy weather. Chronic grass tetany is generally slow to develop and muscular affection may be limited to twitching, a clumsy walk or exaggerated motions , but convulsions may occur if animals are driven or handled roughly (McDONALD et al., 1988).

Why hypomagnesemia is not observed in ruminants on warm season grasses?

There is the explanation; these grasses are low in crude protein and potassium, and higher in magnesium content; grown under a low NPK-fertilizers application (hot weather- water stress is obvious). The main advantages of the grasses are their summer growth habit, providing when temperate (cool) grasses (perennial ryegrass, orchardgrass…) have become semi- dormant , and their ability to grow to use soil moisture efficiently. By the same token, they share the disadvantage of all tropical (warm) or C_4 grasses in that their nutritive quality for livestock is lower than that of temperate (C_3) species. This appears to be related to higher fiber and lower crude protein, and potassium concentrations in the warm season grasses (REID and JUNG, 1982).

It should be noted that grazing cattle preferentially select leaf material and protein consumption would therefore be expected to be markedly higher than indicated by whole plant analysis. It is well established that tropical grasses contain relatively high concentration of fiber and low levels of protein (PAYNE, 1966; BUTTERWORTH, 1967). The fundamental differences in leaf structure (Kranz anatomy) and metabolism of C_4 grasses result in marked differences in composition and nutritional quality of tropical and temperate forages (NORTON, 1982). Environmental conditions exert a strong effect on composition of C_4 grasses result in slower rates of degradation of fiber components in rumen (AKIN, 1986), and lower digestibility by ruminants (MINSON, 1981).

Under tropical or subtropical conditions, pastures based on C_4 grasses are generally considered to provide no more than a maintenance level of nutrition for grazing animals. The effects of temperature on cell wall development were examined by FORD et al. (1979); with increasing temperature , leaf neutral detergent fiber (NDF) concentrations in temperate species perennial ryegrass(Lolium perenne) increased, while in tropical species (Panicum maximum) NDF levels decreased. The decrease was due to a decline in cellulose concentration, while hemicellulose and lignin contents increased. There are clear differences in the concentration of minerals; lower levels of Ca and P, and higher concentrations of Mg, Cu in tropical than in temperate grasses (NORTON, 1982); and K concentrations quite low (mean 1.23% for 378 samples) with a high positive correlation with crude protein(REID and JUNG, 1988)in tropical grasses. The same high positive correlation between K and crude protein were found in temperate forages with highest coefficients in ryegrass forage- Lolium multiflorum (HLÁSNÝ, 1990). Tropical grasses appear to contain higher concentrations of Mg (0.36%) than temperate grasses (0.18%)- according to Norton (1982). However, REID and JUNG (1988) found a mean concentration (in 414 samples) only of 0.16 percent Mg in warm season grasses of Northeast of the USA. It is interesting to note that grass tetany at this area has not been observed in animals on warm season grasses. In balance trials with cattle and sheep fed a range of switchgrass (Panicum virgatum) and big bluestem hays in West Virginia , VONA et al. (1984) found that both animal species remained in positive Mg balance at all stages of maturity of the hays.

In Britain, is there a high incidence of cow´s hypomagnesemia, compared with the

Czech conditions?

We can say, yes; if it is based on the „one“ published example from Northern Ireland (McCOY et al., 1992). There, 513 dairy herds were sampled during the grazing season from March to November 1991, and 1266 suckler herds from March 1991 to February 1992. It was found; blood Mg below 0.8 mmol/l in 14.1% of dairy, and in 33.9% of suckler cows. In addition, the 8.2% of suckler cows had blood Mg below 0.6 mmol/l! The peak of hypomagnesemia incidence , in both dairy and suckler herds occurred in the period from March to June, coinciding with the period of peak milk production.

However, in connection with the BSE incidence epidemiological studies in the UK (to 1993), there is no informations (for example the journal;“Veterinary Record“) about the Mg status in the Britain cows. Unfortunately, the same we can see later; the Mg status did not evaluated, if even in nutritional experiment (perennial ryegrass)- the BSE in cows was introduced (MOORBY et al., 2000). If we compare the period (1986- 1991) of increased incidence of BSE in the UK with the Czech conditions , there was found that;

- the Mg content is lowest, and on the other hand protein content, and the value of K/(Ca+Mg) were found as highest in ryegrass forage (HLÁSNÝ, 1989)
- it is necessary to exceed the supply of magnesium by 60-100% of Czechoslovak Standard Norm, to cow feed rations, which was presented in mathematical expressions for achieve better Mg requirement, in order to reach satisfactory K/(Ca+Mg) values in dairy rations(HLÁSNÝ, 1989a)
- the highest positive correlation coefficients between potasium and crude protein, nitrate content, K/(Ca+Mg) values were found in ryegrass forage, compared with other forages dominated in South Bohemia (HLÁSNÝ, 1990)
- in summer season, tested 53 dairy herds (793 cows) on the eight sort rations , the serum Mg levels averaged: high pregnant- 0.85 mmol/l, early lactation and similarly group 3-4 months after parturition- 0.88 mmol/l. Only if small grain forages or grasses plus green maize were fed, the blood Mg values decreased below 0.8 mmol/l (HLÁSNÝ, 1991)
- therefore it was profitable to use mineral supplement with higher Mg content; as the example when 260 tonnes were used in about 7300 high pregnant heifers - first calving cows, and the significant decrease of newborn calves mortality was observed (HLÁSNÝ,1989b- see also „Medline“).

Can be found to BSE like symptomes-neurodegeneration, in ruminants?

In cases of protracted ryegrass staggers of sheep and cattle, MASON (1968) described cerebellar lesions involving Purkinje cell axons. These lesions consist of eosinophilic homogenous swellings in the cerebellar granular layer , generally located in groups rather than randomly distributed and with a tendency to be more numerous adjacent to the Purkinje cell layer. In general axons with torpedoes were myelinated at least over part of their traceable length, often on both sides of the swelling. Total encasement with myelin was often demonstrable about small swellings, whereas larger ones sometimes had only vestiges remaining. In one early developing lesion axonal rupture had occurred with with retraction. The myelin sheat, hovewer, remained intact both about the bulb and the empty retraction space. The myelin sheats would appear to remain relatively unaffected about degenerating axons. Vacuoles were observed in some torpedoes. It is believed that torpedo development as a reaction of Purkinje cells to simple atrophy, to be caused by disturbances of neurone metabolism. It appears that the longer the disturbing syndromes has been present, the greater the likelihood of finding these axonal changes, in protracted ryegrass staggers in sheep and cattle. Therefore the lesions described are not regarded as pathognomonic of protracted ryegrass staggers but probably arise from a number of factors, which may include disturbed neuronal metabolism, neuronal exhaustion and repeated anoxic insults (MASON, 1968).

The similar histopathological lesions were found in moufflons in „Britain pasture“, with natural cccurence of scrapie (WOOD et al., 1992). The most obvious lesion in the cerebellum occurred in the granular layer, where there was neuronal loss and vacuolation which was sometimes severe and diffuse. Many Purkinje cells were lost or shrunken and there was a marked proliferation and hypertrophy of glial nuclei apparent in the outer part of the granular layer.

Other questions about prion and ammonia magnesium theory

However, also it is possible to explain the „prions theory“ on the ammonia- magnesium „simultaneous“ action principle in ruminants, by following steps:

- high rate and extent of degradation of dietary crude protein causing high concentrations of ammonia-N in rumen results in hyperammonemia, because of diminished capacity of the liver to synthetize urea
- of prime importance (control of the metabolic pathways- ammonia elimination) in the control of carbamoyl- phosphate synthase activity is the mitochondrial concentration of N-acetylglutamate, a compound that is indispensable for enzyme activity
- however, the concentration of mitochondrial free Mg may be relevant, since binding N-acetylglutamate to carbamoyl- phosphate synthase is dependent on this action
- therefore in Mg- deficiency the ureagenesis in liver ornithine cycle is diminished
- the Mg ions has been shown to affect guanine nucleotide binding proteins (G proteins) in several ways:

a/ nanomolar concentrations of Mg ions are required for GPT-ase activity,
micromolar concentrations are required for receptor mediated activation of G proteins

b/ milimolar concentrations of Mg increase the affinity of various types of receptors for agonists , an effect thought to result from increased receptor- G- protein coupling, voltage dependent – Ca channel, and N-methyl-D- aspartate (NMDA) receptor operated ionic channel

- both acute and chronic hyperammonemia are associated with a decreased brain aspartate (and glutamate) concentration:

a/ these amino acids are major neurotransmitters in the mammalian CNS , produce their effects by interacting with specific receptors on the cell surface , the excitatory amino acid receptors

b/ the most well characterized is NMDA receptor which also is permeable to Ca ions

- overstimulation of the NMDA receptor as well as other excitatory amino receptors results in neurotoxicity and neuronal injury .

Finally, there may be the common relationships; abnormal PrP proteins which resist normal protease degradation, and the G-proteins which also can aggregate (low Mg and low aspartate concentrations), especially in neurons in the brain- during hyperammonemia, and Mg-deficiency.

Only spongiform encephalopathy- „kuru“; have an extraordinary mystery. However, there is a question; what cadaverine- putrescine (histamines) have the inhibitory effects on the protease activity on nervous cells surface in cannibals?

According to the ammonia – magnesium theory of BSE we can explain other uncertainties- mystery about the BSE etiology, incidence… as for example :

- why spongiform encephalopathy is observed only in ruminants and carnivorous (some human „individuals“ are also carnivorous) animals ? (because only in ruminants naturally hypomagnesemia- hyperammonemia may be observed; and in carnivora – in cats especially, naturally high intake of meat- protein is obvious)
- why BSE is not observed in countries with hot weather? (because there are conditions for tropical- warm season grasses in ruminants fed)

- why scrapie was first observed in Iceland? (because no legume- only grasses feeding; there are only small grazing areas – therefore high NPK-fertilization by excrements of sheeps; and could weather- therefore the ideal conditions for chronic hypomagnesemia in 18.century, yet…)
- why BSE is observed mostly in „dairy" ruminants? (because of high milk production and disturbances about calcium- magnesium metabolism)
- why BSE is observed only in older cows? (see; the same principle as in hypomagnesemia, etc).

However, only one mystery; „chronology of epidemic of the BSE in the UK (1986-2000)"- can not explain the „ammonia- magnesium theory". So, there is a finally question, especially for Britain nutritionists: what changes (increase?) in Mg- supplementation to britain dairy feed rations were used after 1992/93 years period (after „well known experiment";HLÁSNÝ, 1989b- see „Medline-1992"; in Czech Republic)?

In addition, what is the opinion of american scientist Dr.Paul BROWN?

The consultant to the European CJD surveillance program, and Chairman of the transmissible spongiform encephalopathy advisory committee of the United States Food and Drug Administration, he says: „It is sometimes forgotten that in the story of BSE and variant Creutzfeldt- Jakob disease (vCJD) there is but one incontestable fact, that BSE is the cause of vCJD. First suggested by their temporospatial association and the distinctive and shared biological and molecular features. All the rest is speculation, more or less plausible according to the arguments advanced and the absence of any satisfactory alternative explanations. From an epidemiological point of view BSE has been a classic epidemic and will undoubtebly become a textbook example for students (see chrolology of epidemic of BSE in the UK: 1986-2000). From economic, political, and medical points of view it has been an unmitigated disaster**…** Indeed, the story of BSE and vCJD will, as the inquiry shows, furnish a rich vein of ore to be mined by scientists, governements, and the media when faced with future prospects of epidemic disease in animal or human populations" (BROWN, 2001).

HLÁSNÝ,J.: Some experiences from United Kingdom concerning the history about Mg- research in ruminants; and BSE . Výzkum v chovu skotu(Rapotín), 45, March 2003 (1); 22-34

The BSE was initially recognized in cattle in the UK in 1986; there is good information that it had not occurred before then. Epidemiological research led to the conclusion that the bovine agent had originated from the scrapie agent, which had been present in sheep in the United Kingdom for at last 200 years. It is presumed, but will likely never be proven, that the scrapie agent jumped species and moved into cattle when sheep offal was included in protein supplements fed to cattle.

Ammonia- magnesium theory and the BSE(informations from bulletin (Rapotín); „Výzkum v chovu skotu"- 2001, 2002)

In Britain, perennial ryegrass is the most important species of sown pastures . There the composition of dry matter (DM) of pasture is very variable; for example the crude protein (CP) may reached to over 300 g /kg DM herbage in young , heavily fertilized grass. In contrast, there are no published references to date in which intake of crude protein-potassium high enough to lead to a state of hyperammonemia- hypomagnesemia during the incubation period of the BSE. Therefore it was prompted to air our views in studies of CNS neurotoxities by nutritional causes with the conclusion that the hypomagnesemia plus hyperammonemia „simultaneous" action on the ruminant tissues (the CNS and liver tissue, especially)- and the nervous disease may be involved . As a typical example; the ryegrass staggers can be showed in ruminants. Also, perennial ryegrass feeding must be considered in

the differential diagnosis of hypomagnesemia**.** So, the various clinical symptoms can be observed because the nervous system controlling both voluntary and unvoluntary muscles is affected (Mg and Ca disturbances). It seems, that during the chronic hypomagnesemic disease, the heavy weather changes (cold- rainy, windy...) or nutrition (high intake of crude protein...) stress - these episodes of acute abruptions, may accelerate the nervous, like to „BSE" disease. If the BSE is involved; a longer- chronic action of corresponding biochemical changes in the blood (CSF) is necessary, to rise irreversible neurodegenerative changes (HLÁSNÝ, 2001; 2002).

Decreasing interest about the ruminants Mg-research in the UK from mid-1980s?

(informations from journals; „Animal Science"- 1999; „British Veterinary Journal"- 1995)

Since 1985 , nitrogen application rates to grass have progressively declined. Increasing environmental issues and the present interest in organic farming and low input systems indicate that these trends will continue in the UK. Present overall fertilizer use for grazing on dairy farms is about 170 kg N, 10 kg P and 20 kg K per ha. Higher rates are used for intensive silage production , 200 kg N, 15 kg P and 50 kg K per ha. In contrast recommended applications are 340 kg N, 18 kg P and 25 kg K per ha for grazing and 380 kg N, 40 kg P and 260 kg K per ha for intensive silage. In the critical spring and automn periods, herbage dry-matter intakes may be important as magnesium content and availability when assessing the risk of hypomagnesaemia in the grazing cow. If environmental or economic policies were to result in a reduction in the crude protein content of grazed herbage from ca. 250 to ca. 200 g/kg dry matter , the phosphorus intake of cow giving 30 kg milk per day would fall by 0.5 g/kg dry-matter intake. This together with any desirable additional magnesium, should preferably be given as an oral mineral supplement rather than by attempting to modify the mineral content of herbage (HEMINGWAY, 1999). This author´s summary indicates that in the UK there is the „intention" to use the high N- fertilization (and K-fertilization) for intensive silage production, especially. However, according to the britain Mg-research (see below), at this high fertilizers consumption, the hypomagnesaemia in ruminants is involved. There is the example from the UK ; about „probably" heavy fertilized forage (silage- first cut) feeding. This is the report from MOORBY et al. (2000) which describes a nutritional experiment in dairy cows, as a „different cause of the BSE". These authors , during the last six weeks of the dry period they were offered one of three grass silage (first cut perennial ryegrass)- based diets, offered ad libitum. After calving, all the animals received the same lactation diet consisting of ad libitum access to ryegrass silage. After the 21 weeks of lactation, six from the 47 animals developed clinical signs of BSE, which was later confirmed by histopathological examination. In addition the BSE developed after 27 weeks of ryegrass feeding- without meat and bone meal in the experimental diet; unfortunately, without the Mg status of cows testing…

According to the paper of HEMINGWAY (1999), in the UK there was the greatest „agronomic" Mg-research (in ruminants) in the world; unfortunately only about to the mid-1970s. The article „The effect of changing patterns of fertilizer applications on the major mineral composition of herbage in relation to the requirements of cattle: a 50 – year review"; this shows that the long time research about the NPK fertilization in the UK has been sumarized. In addition, the results based on the literature review; from the long time of high experienced author are important about the „estimate" of Mg-deficit in britain ruminants. According to the list of the article references, there are 28 references; their „title" shows that it is sure; „hypomagnesaemia" (bold writing- references) was studied in britain ruminants. This is ca. one third of the all citated references. However, it is important to emphasize, that 25 of these references were published to mid-1970s and only three were published in the 1996/98 period. Therefore, acording to the study of HEMINGWAY (1999); within twenty

years period (1976- 1996) in the UK, there was not interest about the Mg-research. In addition, within this 20-years period, there is the begining of the BSE incidence in the UK.

Grass tetany , the rapid occurrence of clinical hypomagnesaemia in ruminants when changed from winter diets to grazing young, heavily fertilized grass in spring, is of considerable economic importance in the UK. Since the paper of SJOLLEMA (1930) on hypomagnesaemia in cattle („grass staggers"), now there is greater understanding of the pathogenesis of this disease; yet it is still a source of significant economic loss. Now that it is recognized that the major site of net magnesium absorption is the stratified squamous epithelium common to the ruminant fore- stomachs , a great deal is now known as to the mechanism by which intraruminal potassium ions may reduce the active transport of magnesium ions across the tissue. Therefore the use of potassic fertilizers should be restricted in order to reduce the incidence of grass tetany. However, the phenomenon of individual susceptibility to grass tetany within a dairy herd of similar animals, the so-called indicator cows, still requires a convincing explanation (DUA and CARE, 1995).

According to the paper of these authors, in the UK there was the greatest „veterinary" Mg-research (in ruminants) in the world; unfortunately only to the mid- 1980s.This great Mg-research also shows on the real probability about the „abnormal" Mg-deficit in britain ruminants from 1950s to mid-1980s. In addition, it seems that there is the similarity between the individual susceptibility in the hypomagnesaemic and the BSE „indicator cows"; because the incidence of the BSE within a dairy herd of similar animals is very low- from 0.14 to 3.3% (WILESMITH et al., 1988).

Increasing interest about dietary cattle Mg-supplying in the UK from 1993/94? (informations from journal; „The Veterinary Record")

In britain veterinary journals (period; 1985-1995), there is only one information (article) about the cow hypomagnesaemia testing(McCOY et al., 1993)- from Northern Ireland (clotted blood samples submitted under the Brucellosis Eradication Scheme were used for this survey). There, 513 dairy and 1266 suckler cow herds were sampled during the grazing season from March to November 1991 (to February 1992- suckler cows). It was found; serum blood Mg below 0.8 mmol/l in 14.1 of dairy and in 33.9% of suckler cows. The peak of hypomagnesaemia incidence; in both dairy and suckler herds occurred in the period from March to June. In addition , in 8.2% of suckler cows – the blood Mg below 0.6 mmol/l was found !

One year later, report from McCOY et al. (1994) describes a novel method to evaluate the most popular commercially available hardened magnesium blocks – as oral mineral Mg-supplement in cattle feeding. There are following informations (from their survey- article); about the evidence of Mg- oral supplementation changes in the UK, in cattle:

Proof No 1: In early 1980s (1983; published in „Outlook on Agriculture"), it was stated that in most circumstances there is no safe alternative to providing extra dietary magnesium (Mg), with 30 g of available Mg per lactating cow per day being an average target.

So, there is the evidence about the Mg-deficit in britain cows in 1980s yet, because - high Mg-supplementation was recommended (for example; in a cow with 20 kg dry matter (DM) intake; 30 g only as extra dietary Mg = 0.15% Mg of DM of the dairy ration

Proof No2: The incidence of hypomagnesaemia in adult cows remains high in Northern Ireland. An increasingly popular method of supplying additional dietary magnesium is by allowing cattle free access to hardened, magnesium- rich blocks or licks.

So, there is the evidence that the problem about Mg-deficit in britain cows; not only continued to 1993/94 period ,but at this time (period) there is increased „preventive" interest to cow

Mg supplying .

Proof No 3: Five commercial hardened Mg-blocks were tested in non-pregnant heifers with the intention to achieve intake of 30 g as extra dietary Mg; per animal and day (during experiment, it was found; 4.4 g, 58.3 g, 8.7 g, 49.2 g, 15.0 g Mg- on 1,2,3,4,5 types of Mg blocks, resp.). The present study shows that available commercial Mg-blocks; have considerable variation in palatability, Mg-content and Mg-intake of the hardened Mg rich blocks – as it was concluded by authors.

However, there is evidence; if some of commercial Mg-blocks (type 2 and 4) are used , very high Mg intake is achieved.

Proof No 4: Many magnesium rich blocks are available, but unfortunately little is known about their relative effectiveness. There is a need for commercially available blocks to by evaluated before being put on the market. The use of time-lapse video recording (equipment) provides an additional method of carrying out such quality control (McCOY et al.,1994).

So, the begining (1993/94) of the „special" interest to monitoring of intake Mg-blocks by cattle; this also is evidence of higher interest of Mg-supplying in the UK

So together, finally; there is evidence that in 1993/94 period; it seems that in the UK it was the begining about; an increasingly popular method of supplying additional dietary magnesium- preferably be given as an oral mineral supplement rather than by attempting to modify the mineral content of herbage- by Mg fertilization.

SUMMARY

It can be concluded, that in the UK there were ideal conditions for hypomagnesaemia-hyperammonemia status in ruminants, during the period of highest BSE incidence.

In Britain praxis, higher rates were used for intensive silage production, especially; 200 kg N, 15 kg P and 50 kg K per ha. In contrast recommended applications from Ministry of Agriculture, Fisheries and Food (1994) were higher; 380 kg N, 40 kg P and 260 kg K per ha for intensive silage. There is evidence at example from HEMINGWAY (1999), that in Britain there is the intention to use high rates of N-fertilizers, especially; therefore hyperammonemia in ruminants can be involved..

In the UK, there was not interest about the Mg-research in ruminants within twenty years period (1976- 1996). In contrast it can be interpret , acording to the study of HEMINGWAY (1999); that to mid-1970s, in the UK there the Mg research it was greatest in the world. In addition, within this 20-years period, there is the begining of the BSE incidence in the UK.

Also, according to the DUA and CARE (1995) , in the UK there was the greatest „veterinary" Mg-research (in ruminants) in the world; unfortunately only to the mid- 1980s. This great Mg-research also shows on the real probability about the „abnormal" Mg-deficit in britain ruminants from 1950s to mid-1980s . From both publications, there is evidence about reality of Mg-deficit also in the next decade years (1985-1995) in britain ruminants. However, with the high probability that after significant increase of crude protein in dairy rations (NRC,1985; publication „Ruminant Nitrogen Usage") – in mid- 1980s, it was without equality of oral Mg-supplementation. However, after 1993/94 period, there are some evidence about the additional dietary Mg-supplementation increase (McCOY et al., 1994).

Therefore if we will put into practice „ammonia- magnesium theory" in Britain cows; **significantly higher dietary Mg-supplementation - can be a cause about the BSE incidence decrease, after 1993/94 period.**

Conclusion about the scenario of BSE beginning in the UK and the decrease of the BSE after 2002 in other states of the EU

A / I concluded following scenario („Výzkum v chovu skotu“, December 2002) that;

Why the BSE began in the United Kingdom ?

a/ According to the scientific „official“ theory:

The BSE was initially recognized in cattle in the UK in 1986; there is good information that it had not occurred before then. The theory favoured by most scientists who have studied the disease is that it originated from an infection by scrapie in sheep. It began in the UK and not elsewhere because of a comparatively **high incidence of scrapie in UK sheep and a comparatively large proportion of sheep in the mix of carcases rendered** for animal feed for livestock.

b/ According to the alternative „BSE ammonia- magnesium“ theory:

It began in the United Kingdom (UK) because of there the **„ideal“ rainfall, and the available water capacity of the soil for high NPK fertilizers utilization** by grassland (mostly ryegrass). This is a major determinant for high grasses yield ranging in the UK from 6000- 14000 kg DM/ha under intensive fertilization …(more about this; **see article above mentioned).**

B / Later, I concluded following scenario („Výzkum v chovu skotu“, March 2003 (http://www.nal.usda.gov/awic/pubs/bsebib.htm)) that ;

1. In the UK there were ideal conditions for hypomagnesaemia- hyperammonemia status in ruminants, during the period of highest BSE incidence.

Why?

- higher rates of the NPK were used for intensive grassland production (nitrogen especially); 200 kg N, 15 kg P and 50 kg K per ha (1980s); therefore hyperammonemia in ruminants can be involved..
- according to the DUA and CARE (1995) and the study of HEMINGWAY (1999) , in the UK there was the greatest „veterinary“ Mg-research (in ruminants) in the world; unfortunately only to the mid- 1980s
- this great Mg-research also shows on the real probability about the „abnormal“ Mg-deficit in britain ruminants from 1950s to mid-1980s . From both publications, there is evidence about reality of Mg-deficit also in the next decade years (1985-1995) in britain ruminants.
- however, with the high probability that after significant increase of crude protein in dairy rations (NRC,1985; publication „Ruminant Nitrogen Usage“) – in mid- 1980s, it was without equality of oral Mg-supplementation.

2.So, it was „mysteriously concluded“ about the „BSE infectiosity“ (as a key argument; about in 1987 the ban of the MBM feeding in the UK ruminants) ; five years incubation period.

Why five years? (according to ammonia- magnesium theory)

- after 1993/94 period, there are some evidence about the additional dietary Mg-supplementation increase (McCOY et al., 1994)
- therefore if we will put into practice „ammonia- magnesium theory“ in Britain cows; **significantly higher dietary Mg-supplementation - can be a cause about the BSE incidence decrease, after 1993/94 period.**

3. In the period 2001/2002 the BSE „incidence picked" in other states of the EU - after „common BSE testing in 2001" (the exceptioon is „green Ireland")
(http://home.hetnet.nl/~mad.cow/)
However, it was **„mysteriously"** found that this is as a result about the ban of meat and bone meal (MBM) feeding during 1990s.

Why BSE incidence decreased in 2003- in other states of the EU ? (according to the „BSE ammonia- magnesium theory")

According to the NRC (1989)- it was a begining about high protein level (19%) recommended to dairy rations, however only during first three weeks (0-21 days postpartum) after calving. However, the same high protein recommendations (18.8 % dry matter) was recommended from McCULLOUGH (1994) to dairy rations of high producing „supercows"- during the „all" lactation. So, above mentioned recommendation (NRC 1989) were **„overdosed" in the „world dairy practice"**. For example, the same high protein level is recommended in turkeys- in animals with highest protein requirements from animals (NRC,1994). In growing young turkeys (age; 11 to 14 weeks) there is the recommendation (http://www.nap.edu/books/0309048923/html/) 19 percent of protein of diet (90% dry matter). Almost the same situation is in „monogastric" young rapidly growing pigs allowed ad libitum diet of 90% dry matter (NRC, 1998)- average weight in range 15 kg (20.9% of crude protein) and 35 kg (18% of CP) (http://darwin.nap.edu/books/0309059933/html). However, the dairy research **(during 1990s) resulted to decrease of protein** content in dairy cows rations (compared to the NRC,1989) - see Nutrient Requirements of Dairy Cattle; Seventh Revised Edition, 2001 (http://www.nap.edu/catalog/9825.html). So, as in the early of 1990s – there was the begining about higher dietary Mg-supplementation; the same was about the lower dietary protein-supplementation – in dairy cow rations.!

Generally, this „BSE scenario" can be confirmed by the repetition of „nutritonal experiment" performed in the United Kingdom (BSE is not an „infectious" disease)- see publications; MOORBY et al., 2000; DEWHURST et al.,2000; MOORBY et al., 2000a;

MOORBY,J.M.- DEWHURST,R.J.- TWEED,J.K.S.- DHANOA,M.S.: Aspects of the metabolism of dairy cows during the incubation period of bovine spongiform encephalopathy. Vet.Rec., 147, 2000; 409-412

DEWHURST,R.J.- DEWHURST,R.J.- MOORBY,J.M.- DHANOA,M.S.- EVANS,R.T.- FISHER,W.J.: Effects of altering energy and protein supply to dairy cows during the dry period. 1. Intake, body condition, and milk production. J.Dairy Sci., 83, 2000: 1782-1794.

MOORBY,J.M.- DEWHURST,R.J.- TWEED,J.K.S.- DHANOA,M.S.- BECK,N.F.G.: Effects of altering energy and protein supply to dairy cows during the dry period. 2. Metabolic and Hormonal Responses. J.Dairy Sci., 83, 2000a: 1795-1806.

Other supporting literature sources- as a „suspicion" that meat and bone meal (MBM) was only „occasionally fed" in Britain cows

A. Conclusion from „Animal Nutrition" and from „Internet statements";

1. Meat products are more valuable for simple- stomached than for ruminant animals, since the latter do not require a dietary supply of high quality protein. Both meat meal and MBM are eaten readily by pigs and poultry, and may be given at levels of up to 150 kg/t of the diet for laying hens and young pigs…, **these products are not readily acceptable to ruminants,**

and must be introduced into their diets gradually. Meat meal generally contains from 600 to 700 g/kg of protein compared with about 450 to 550 g/ kg for MBM. Therefore, considerable care is required in storing the meat products to prevent the development of rancidity… (McDONALD et al., 1988).

2. At the time that feed producers switched from soya bean meal to meat and bone meal, Britain farmers buying compound feed **would not know what ingredients** had been used to provide protein. **He would not know if the protein source in the compound was soya bean meal or meat and bone meal**. The arguments for a declaration of ingredients are well rehearsed in an NFU paper prepared in March 1983. The absence of ingredient listing meant that farmers buying compound feedstffs would not know whether or not the feed included meat and bone meal, and if so, whether it was bovine or ovine meat and bone meal… The alternative to purchasing compound feeds is for a farmer to purchase the individual ingredients (referred to as „straights") and to prepare feed compounds himself…" (GILL, 1998)

3. Submission of BSE cases and samples was based on a voluntary commercial decision of the attending veterinary surgeon. **The Scientific paper published in the Veterinary Record of October 1987 was the first large scale publicity that the profession received.** The surveillance of BSE up to June 1988 and indeed the surveillance of any non-notifiable disease is based upon the vigilance and inquisitiveness of the farmer and attending veterinary surgeon, with the voluntary referral to a VI Centre. The system is informal and haphazard and is based upon a relationship between the practice and the VI Centre that may or may not exist (SIBLEY, 1999).

References

McDONALD,P.- EDWARDS,R.A.- GREENHALGH,J.F.D.: Animal Nutrition (4th edition), **London 1988: 543 pages**
The BSE Inquiry; statements;
Statement of Ben GILL, 1998 (www.bseinquiry.gov.uk/files/ws/s047.pdf).
Statement of Richard SIBLEY, 1999 (**www.bseinquiry.gov.uk/files/ws/s421.pdf**).

B. Some details about „Internet statements";

The BSE was initially recognized in cattle in the UK in 1986 (November); there is good information that it had not occurred before then (official and scientific statements!):

1. **However, there is anecdotal evidence to suggest that there may have been earlier, undiagnosed cases of BSE.**
Various farmers and veterinarians gave evidence of seeing cows in the early to mid-1980s with similar symptoms to those of BSE (www.bseinquiry.gov.uk/report/volume3/chapter.htm)

Statement of Mr Brian AHERN of Church farm (28/09/1998); he says: „I began farming 120 acres here in 1977 with a dairy herd of 45 cows. From 1981 onwards the heifer calves were reared as dairy herd replacements 15 miles away. In March 1985 I had two cows showing early symptoms of what we now know to be BSE. I called out my local private practice vet and treated them for hypomagnesaemia as he advised. **The condition of one cow became so bad in the late summer of 1985 that she was slaughtered…** The other cow showed BSE symptoms in early 1985 was also put down soon afterwards for humane reasons when it

became clear that her condition was still getting worse. (www.bseinquiry.gov.uk/files/ws/s129.pdf).

Statement of Mr Eric COMLEY (28/09/1998); he says:
„Sometime in early 1980s a young cow who had been treated with the rest of the herd with organophosphate (OP) pour on to eliminate the warble fly pest came in from the field for the afternoon milking with an unusual unsteadiness on her legs. Later on in the 1990s we had another cow with similar symptoms , this time diagnosed as BSE. We lost 8 or 9 cows with this complaint. I became suspicious of the OP pour on treatment in the late 1980s and stoped using it…". (www.bseinquiry.gov.uk/files/ws/s132.pdf).

Letter from Dr.Raymond Wiliams to University of Bristol Veterinary School (11/02/1985); he says: „With reference to the above cow, I am writing to briefly describe the clinical syndrome seen over the last two years on Mr Lysley´s two dairy units. Four cases have been seen in total, Two cases last year became so inco- ordinated that they fell down on several occasions and were eventually slaughtered. Nervous acetonaemia and hypomagnesaemia were thought to be the most likely causes, but blood glucose, beta-hydroxy butyrate and calcium/magnesium levels were normal… The present case, No 36, was first examinated a fortnight ago with inco- ordinated gait, with loss of yield and body condition, although appetite was reported reasonable. The cow calved for her sixth lactation on 2 November 1984. If a post- mortem is necessary, **Mr Lysley would be very grateful if the carcase can be used for human consumption….**".
www.bseinquiry.gov.uk/report/volume3/chapter.htm.

Statement of Dr.David BEE (february, 1998), veterinary surgeon in private practice; he says:„ I saw cases of BSE from December 1984 to the present. Over the period December 1984 to September 1985 our Practice was called on many occasions to Mr.Stent, Pitsham Farm, Midhurst to see cattle with a variety of unusual clinical manifestations…" (in total; 13 cows was died and examined).(www.bseinquiry.gov.uk/files/ws/s006.pdf)

2. Submission of BSE cases and samples was based on a voluntary commercial decision of the attending veterinary surgeon?

Statement of Richard SIBLEY, chairman of the BCVA BSE Group (25/05/1999), he says: „The British Cattle Veterinary Association is a specialist division of the British Veterinary Association. We have 1600 members of whom 1000 are practising veterinary surgeons working with cattle in farm animal veterinary practices. It was the inquiring minds and instinctive concerns of some veterinary surgeons that led to them to refer the first BSE cases to such organisations as the Veterinary Investigation Service who investigated further. Such referral was voluntary and based upon a relationship between the private practice and the local VI Centre. Not all practices submit samples or refer cases to their local VI Centre. Private laboratories provide a similar and competitive service. We now know that most BSE cases occur as a single sporadic case and many of these would not have been referred to any VI Centre. Submission of cases and samples was based on a voluntary commercial decision of the attending veterinary surgeon. It may be that the original geographical recorded incidence of this disease was influenced by the vigilance and activity of veterinary surgeons in those areas and the relationship they had with their local VI Centre. **The Scientific paper published in the Veterinary Record of October 1987 was the first large scale publicity that the profession received.** The surveillance of BSE up to June 1988 and indeed the surveillance of any non-notifiable disease is based upon the vigilance and inquisitiveness of

the farmer and attending veterinary surgeon, with the voluntary referral to a VI Centre. The system is informal and haphazard and is based upon a relationship between the practice and the VI Centre that may or may not exist. The relationship is often dependent on personal contact, commercial expediency and local availability rather than the wider ramifications of national disease surveillance. The epidemiological studies of the first cases that concluded that an agent was transmitting the disease contained in meat and bone meal (MBM) provided the key to control. The control measures introduced were logical and rational as well as inspirational. Veterinary practitioners are scientists who meet practical disease control challenges on a daily basis. Such veterinary surgeons have much to offer in terms of BSE surveillance and control, including determining practical policies. **However, there was scant opportunity for their voices to be heard with little formal consultation or involvement with policy taking**…".(www.bseinquiry.gov.uk/files/ws/s421.pdf).

3. In the UK the second half of the 1970s and beyond, farmers were being encouraged to increase their milk production. However, where „unpalatable„ MBM was fed in cows?

Statement of Ben GILL (30/04/1998); farmer, with a beef, sheep and arable farm; he says: „I have been involved in the emerging story of BSE since the NFU first learnt of the existence of a nine cattle in July 1987. I was vice chairman and chairman of the Livestock and Wool Committee of the NFU (1986-1991). Feed compounds used for feeding cattle, farmers may buy compound feed from feed producers. The actual ingredients used will vary from time to time and from producer to producer. These are commercial decisions taken by the feed producers. For example, protein could amongst others be generated by soya bean meal, or from processed meat and bone meal. At the time that feed producers switched from soya bean meal to meat and bone meal, there would have been no restrictions on them doing so. A farmer buying compound feed would not know what ingredients had been used to provide protein. **He would not know if the protein source in the compound was soya bean meal or meat and bone meal**. The arguments for a declaration of ingredients are well rehearsed in an NFU paper prepared in March 1983. The absence of ingredient listing meant that farmers buying compound feedstffs would not know whether or not the feed included meat and bone meal, and if so, whether it was bovine or ovine meat and bone meal… The alternative to purchasing compound feeds is for a farmer to purchase the individual ingredients (referred to as „straights") and to prepare feed compounds himself…" (www.bseinquiry.gov.uk/files/ws/s047.pdf).

During the early 1980s, britain farmers were looking to increase their milk yields significantly. Improved animal feeding, improved forage conservation, the milk yield of dairy cows over this period of time went up by 25 per cent. Up until 1984, there were no quotas on milk production, so that meant farmers were at liberty to really pump up production as much as they liked. During the 1980s- of this increasing milk yield, most farmers went from shipper and buyer type feeding to one of parlour- type. **Dairy animals during this period of time probably spent no more than ten minutes in the parlour**. So, the speed of eating was quite important, hence the reason for making sure that the industry produced a consistent feed that was eaten and taken readily by the dairy cows in the parlour (Mr Robert Thompson, Commercial director- Agricultural Co-operative; 28 April 1998)

For cattle, particularly, MBM was not palatable ingredient. The **maximum was around about 5 per cent in concentrates, because increased the amount of MBM beyond 5 per cent, the cow would start to find it unpalatable** (Dr.Martin Clark, nutritionist- Midland Shires Farmers; 28 April 1998). The concentrates were quite expensive products. Where they were fed straight to dairy cows it was particularly where dairy farmers segregated high yielding undegradable protein (UDP).. In the UK the second half of the 1970s and beyond,

there was quite a long period of time, certainly ten years, during which dairy farmers were being encouraged to increase their milk production**. Meat and bone meal (MBM) was sold to animal feed compounders as a source of protein in Wales as in England, there were three large national feed companies.** The rendering industry provides a service by collecting and processing all animal material from slaughterhouses, cutting plants and butchers´ shops, which is not used for human consumption. (www.bseinquiry.gov.uk/report/volume9/chapte24.htm).