Bovine spongiform encephalopathy, epidemics that never was and Alzheimer´s disease connection?

In 2020, we pointed out in the journal "Výzkum v chovu skotu" (three publications) that bovine spongiform encephalopathy (BSE) is not an infectious but metabolic origin (magnesium deficiency...) disease. In this context, attention was also drawn to the fact that there is a link between bovine BSE - magnesium deficiency and Alzheimer's disease in humans. In recent years, the issue has similarly been highlighted in a number of publications, such as the following;

Turkey, 2007; Serum magnesium level and clinical deterioration in Alzheimer's disease
https://pubmed.ncbi.nlm.nih.gov/17992016/
China, 2011; Magnesium in Alzheimer’s disease
https://www.ncbi.nlm.nih.gov/books/NBK507256/
China, 2013; Magnesium Status in Alzheimer’s Disease: A Systematic Review
https://pubmed.ncbi.nlm.nih.gov/23658180/
Australia, 2014; Dietary Mineral Intake and Risk of Mild Cognitive Impairment: The PATH through Life Project
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3912433/
USA, 2015; Magnesium Status in Alzheimer’s Disease: A Systematic Review
https://journals.sagepub.com/doi/pdf/10.1177/1533317515602674
Reaction to the USA;
a/ USA, 2015; Magnesium and Alzheimers
https://www.clearvuehealth.com/d/magnesium-alzheimer/
b/ USA, 2020; Magnesium as a nutrient
https://www.clearvuehealth.com/d/magnesium-alzheimer/
USA, 2016; Magnesium
https://www.alzdiscovery.org/uploads/cognitive_vitality_media/Magnesium-Cognitive-Vitality-For-Researchers.pdf
USA, 2017; Magnesium Sulfate for the Improvement of Cognition in Patients With Alzheimer Disease. https://clinicaltrials.gov/ct2/show/NCT03038334
Netherlands , 2017; Serum magnesium is associated with the risk of dementia
https://pubmed.ncbi.nlm.nih.gov/28931641/
Reaction to the Netherlands ;
a/ USA, 2017 ; FROM THE WEBMD ARCHIVES, By Steven Reinberg
https://www.webmd.com/alzheimers/news/20170920/high-low-magnesium-levels-tied-to-dementia-risk
b/ India, 2017; Serum magnesium and all-cause dementia including Alzheimer disease,
https://n.neurology.org/content/serum-magnesium-and-all-cause-dementia-including-alzheimer-disease
c/ USA, 2017; Too much or too little magnesium can raise dementia risk
https://www.medicalnewstoday.com/articles/319481

d/ UK, 2017; Blood magnesium levels linked to dementia.
https://www.alzheimersresearchuk.org/blood-magnesium-levels-linked-dementia-risk/
USA, 2018; The Role of Magnesium in Neurological Disorders
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6024559/
USA, 2018; The Role of Magnesium in Neurological Disorders
China, 2019; Relations of magnesium intake to cognitive impairment and dementia among participants in the Women's Health Initiative Memory Study: a prospective cohort study
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6858129/
USA, 2020; Magnesium Supplements May Reverse Memory Loss in Alzheimer's Patients
https://universityhealthnews.com/daily/memory/the-best-magnesium-supplement-for-reversing-memory-loss-in-alzheimers/
USA, 2020; Low Serum Magnesium is Associated with Incident Dementia in the ARIC-NCS Cohort https://www.mdpi.com/2072-6643/12/10/3074
Italy,2020; Intra-erythrocytes magnesium deficiency could reflect cognitive impairment status due to vascular disease: a pilot study. https://pubmed.ncbi.nlm.nih.gov/33272305/
Czech Republic, 2020; Is magnesium deficiency the cause of neurodegeneration in animals and humans? 2nd part; Alzheimer´s disease and BSE magnesium- ammonia theory connections.
https://www.researchgate.net/publication/344361915_Is_magnesium_deficiency_the_cause_of_neurodegeneration_in_animals_and_humans_2nd_part_Alzheimers_disease_and_magnesium-_ammonia_theory_connections
Israel, 2020; Intra-erythrocytes magnesium deficiency could reflect cognitive impairment status due to vascular disease: a pilot study, https://pubmed.ncbi.nlm.nih.gov/33272305/
Israel, 2020; Association Between Serum Magnesium Levels and Alzheimer's Disease or Mixed Dementia Patients: A Population-Based Retrospective Controlled Study
https://pubmed.ncbi.nlm.nih.gov/33163901/
USA, 2021; Magnesium and Micro-Elements in Older Persons
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC8001940/
Spain, 2022; Prospective association between dietary magnesium intake and physical performance in older women and men. https://link.springer.com/article/10.1007/s00394-022-02808-z
China, 2022; Association of Circulating Magnesium Levels in Patients With Alzheimer's Disease From 1991 to 2021: A Systematic Review and Meta-Analysis
https://www.frontiersin.org/articles/10.3389/fnagi.2021.799824/full
China, 2022; Magnesium-L-threonate exhibited a neuroprotective effect against oxidative stress damage in HT22 cells and Alzheimer's disease mouse model. 2022
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC8968501/
USA, 2022; Association between magnesium intake and cognition in US older adults: National Health and Nutrition Examination Survey (NHANES) 2011 to 2014 https://alz-journals.onlinelibrary.wiley.com/doi/full/10.1002/trc2.12250

Content

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https://www.vuchs.cz/main/bulletin/vyzkum-v-chovu-skotu-2-2019/

My knowledge about meat and bone meal (MBM) feeding is based on findings from a long-term experience in the field conditions. After five years (1969- 1975) as a veterinary surgeon (large animals) I worked as a veterinary inspector for farm animals nutrition, in South Bohemia (1975- 1990). On many occasions I found, that cows have ceased to receive the mixed feed, which was manufactured and intended for poultry, when contains MBM. It did so in error in the mixing feed company.

In 1991, as assistant professor from the Brno Veterinary University, I worked almost for a year, with an international team at West Virginia University. There I from the journals found that in Britain new disease bovine spongiform encephalopathy(BSE) occurs. To my great surprise, British vets diagnosed it

(Veterinary Record, December1988) as the cause of the disease MBM, which was in Britain reportedly fed in cattle for several years. It was suggested that exposure began in 1981/82 and that the majority of affected animals became infected in calfhood. Then I said, it is interesting; in Czechoslovakia cows do not eat MBM and British cows yes?

1988- 2000; First epidemiological field BSE studies in the United Kingdom (UK) and ten years later, experimentally initiated BSE, without MBM feeding

In 1987/88 there 156 confirmed BSE cases in 145 cattle herds (with at least one confirmed case), and „hypothetical“ was concluded, that BSE has an origin in MBM feeding, when majority of cows become infected as the newborn calves. Based on the computer simulation models, see only 1-2 cases BSE/ herd. So, not a classical infectious epidemy, without experimental confirmation of MBM in cows-calves feeding . This first epidemiological study was constructed in intention determine the hypothesis that BSE is caused by a transmissible agent (Wilesmith et al., 1988).

NOTE; In this field study, which became the basis for infectious "BSE – scrapie – vCJD" hypothesis (to date!), there no information about MBM feeding. In addition, newborn calves would die, when MBM was fed !

At the same time (1988- 1990), in Northern Ireland – no feeding MBM in cows was found, examined by Wilesmith´s team. They concluded that the findings were consistent with the current hypothesis, that affected cattle had been exposed to a scrapie-like agent via cattle feedstuffs containing ruminant-derived protein. However, a preliminary investigation of the potential sources of infection for cattle in Northern Ireland did not provide any conclusive evidence (Denny et al. 1992).

The same was found, when fourteen cases of BSE were diagnosed on the basis of clinical examination in a closed herd of British Friesian cows, during a 9-month period from October 1987 until June 1988. No protein of animal origin had been fed to either heifers or cows, in this herd during the past 5 years and there had been no direct contact with sheep. The herd consisted of 500 cows, the average lactation yield was 5500 litres in 305 days (Winter et al., 1989).

Ten years later, the BSE disease was tested in dairy cows,"nutritonal experiment" performed in England, see three publications (Dewhurst et al., 2000; Moorby et al., 2000; Moorby et al., 2000a). This experiment was conducted using diets and other conditions typical of north western Europe, under well defined conditions of husbandry and nutrition, without MBM

feeding! The effect of altering the amount of protein and energy over the final 6 wk of the dry-period diet, and during the first 21 wk of the subsequent lactation was investigated, in 47 dairy cows. Perennial ryegrass silage was used ad libitum plus a concentrate with high crude protein (CP) level (22.5%). High levels of plasma urea-N were found during lactation and also during dry period. However, after the collection of the last blood sample (21 wk of lactation), six of the 47 animals developed clinical signs of BSE. So, after long-term (28 weeks) dietary protein surplus (18- 20% of DM) was fed, when only 15% of protein in feed ration was needed, and ca 13 percent of dairy cows developed BSE! Such a high percentage of BSE disease was never found, under normal conditions in none of British cow herd.

NOTE; The findings about BSE in the UK, confirm my experience, that cows (cattle) do not eat MBM , even if they have been diagnosed with BSE!

1988- 2012; The occurrence of BSE in countries of European Union

After 2012, the incidence of BSE in the world decreased significantly (according to OIE statistics), with no BSE detected in the UK in 2016, there only one BSE case was detected in France and Spain. Through the end of 2012; 184,621 cases of BSE (mostly to 2000; 180,845 cases) had been confirmed in the United Kingdom (UK), in more than 35,000 herds, BSE peaked in 1992 (37,280 cases). In 2001, the EU introduced compulsory testing on BSE. As the table shows, until then was greater incidence of BSE detected only in the UK, Ireland, Switzerland, Portugal, France. Conversely, from 2001 there is a beginning of BSE incidence in other EU countries, see in particular Spain.

European countries; about the highest incidence of BSE

	to 2012 BSE cases	**to 2000 BSE cases**	**"BSE peak" year/ cases**
UK	184, 621	180,845	1992/ 37 280
Ireland	1,653	442	2002/ 333
Portugal	1,082	159	1999/ 159
France	1,015	80	2001/ 274
Spain	785	0	2003/ 167
Switzerland	464	335	1995/ 68
Germany	412	6	2001/ 125
Italy	144	2	2001/ 48
Belgium	133	19	2001/ 46
Netherlands	80	8	2002/ 24
Poland	73	0	2005/ 19
Czech Republic	30	0	2005/ 8

The table also shows that in addition to UK and Ireland; also in Switzerland in the mid-90th years, has been the high incidence of BSE. In addition; were also cows in Switzerland fed infected MBM, originating from the UK?

1995 - 1996; The discovery of the human form of BSE, new variant CJD (vCJD)

In 1995 and early 1996, a small number of cases of CJD with a remarkably early age at death (29 years) were identified in the UK. With an unusual clinical and pathological phenotype for CJD, including extensive deposition in the brain of florid plaques. British scientists have found two cases of sporadic CJD in teenagers and in a dairy farmer published in The Lancet (October, 1995). However, scientists emphasized that it is necessary to have definitive experiments, to establish whether BSE can transmit to humans. So, it is necessary act quickly, there is a risk (for the government), threatens reparative legal proceedings (Almond et al.,1995).

Therefore American scientist Paul Brown wrote (25 November 1995) in British Medical Journal (BMJ) as follows; „With respect to the four farmers, it is also true that each had at least one infected cow in his herd, raising the possibility of contact infection from the cows or even inhalant infection from the contaminated meat and bone meal feed that caused their illness… Finally, there does not seem to be any need for new governmental hearings, committee meetings, or parliamentary debates about what more might be done because the precautions taken some years ago to eliminate potentially infectious products from commercial distribution were both logical and thorough. We are left looking at possible present consequences of past events over which we now have no control, and we can only hope that the affair will be happily resolved. At least we do not have to face the spectre of reparative legal proceedings, which in this case would amount to a class action suit for anxiety brought by the entire British population against its own government" (ALMOND et al., 1995).

After this "published" threat, pointing to a possible punishment for the British government, the neurologist R.G. Will (National Creutzfeldt-Jakob Disease Surveillance Unit in Edinburgh) wrote the following letter to British neurologists, in just four months (journal Lancet; March 21, 1996); "In the past few weeks we believe we may have identified a new clinico-pathological phenotype of CJD which may be unique to the United Kingdom… COULD YOU PLEASE NOTIFY THE CJD SURVEILLANCE UNIT OF ANY SUCH CASE WITH THIS CLINICAL OR NEUROPATHOLOGICAL PROFILE,

WHETHER OR NOT THE PRESENTATION IS IN THE YOUNGER AGE GROUP? COULD YOU ALSO CHECK YOUR RECORDS AND NOTIFY ANY SIMILAR CASES THAT MAY IN RETROSPECT FIT WITH THIS CLINICOPATHOLOGICAL PROFILE? Four of the recently identified cases were confirmed by brain biopsy. If you are considering brain biopsy in any suspect cases of CJD it is essential to follow the Department of Health guidelines which state that neurosurgical instruments used on any case of CJD must be destroyed and not reused… The identification of a form of CJD that might be casually linked to BSE will result in widespread anxiety and concern..."

Then, even faster (in a few days), on April 6, 1996, in the scientific journal Lancet (Will et al. 1996) was published a "discovery" of a new disease (new variant of CJD) when it was found that a total of 10 people (in words; ten people!) could be infected with beef. Immediately afterwards, in May 1996, not only in Britain but throughout the world began to spread the news of the vicious and incurable disease transmitted to humans.

1996- 2006; Many scientists have tried to legalize BSE / vCJD infection.

However, the predicted “vCJD epidemic” disappeared; so began research about blood transfusion and surgical instruments infection In the UK after finding BSE in 1986 (in 1987, 446 cases) in the previous period the population probably has consumed hundreds (thousands?) of BSE affected animals (including nerve tissue). However, after about 30 years later, the infections of new variant CJD (vCJD) in the UK disappeared, when in 2000 there this disease peaked (28 cases). How can be this "phenomenon" explained?

The incidence of vCJD disease was (UK) from 1995 following; 1995 (3 cases vCJD);1996 (10);1997(10);1998 (18);1999 (15); 2000 (28); 2001 (20); 2002 (17); 2003 (18); 2004 (9); 2005 (5), 2006 (5); 2007 (5); 2008 (2); 2009 (3); 2010 (3); 2011 (5); 2012 (0); 2013(1); 2014-15 (0); 2016 (1); 2017-19; zero cases vCJD); TOTAL (176 cases of vCJD). In the all world media, there is published number of 178 cases, in fact it was only 123 neuropathology confirmed cases. Next supporting evidence (gradually to 2003) about BSE and vCJD transmission is mostly related to description in high-ranking journals (Lancet, Nature, Science, PNAS…).

In 2010, two articles were published (journals; Pract. Neurol. and Lancet) with a question mark, see the following article titles; Variant CJD: where has it gone, or has it? Variant or sporadic Creutzfeldt-Jakob disease? These last two

articles (2010) suggest that the entire scientific saga of BSE transmission to humans has questionable foundations, and that both CJD diseases are not infectious, because they occur only sporadically. In addition, this BSE/ vCJD saga originated and finished in the scientific journal Lancet.Until 2003, about 20 publications in scientific journals were written on this topic, the most frequent authors were; RG Will, Cousens SN, Ironside JW, PG Smith and RS Knight.

However, last mentioned Professor Knight apparently contributed to another "discovery", concerning the transmission of the disease (vCJD) by blood transfusion. However, this could be a confusion of diagnosis (change CJD to vCJD diagnosis ?), as a “detective story”. This follows from the text of the article in The Telegraph (Lambert, 2010) as follows; “ Judy Kenny´s husband Deryck, died of vCJD on October 24 2003. He was the first person recorded to die from the disease contracted via a blood transfusion in the UK... In November 2003, Judy received a phone call from the hospital to say that Deryck’s death was due to the sporadic form of CJD. But then Prof Richard Knight, consultant clinical neurologist at the National CJD Surveillance Unit in Edinburgh, telephoned her and asked to meet. He revealed that Deryck’s death was due to vCJD – and that he had probably contracted the disease from contaminated blood given in a transfusion during his prostatectomy...”

And again very quickly (February 7, 2004) his “discovery” was published (again in The Lancet) as follows; “One of these recipients (Deryck) was identified as developing symptoms of vCJD (65 years) after receiving a transfusion. However, the age of the patient was well beyond that of most vCJD cases (Liewelyn et al., 2004)“.

Almost immediately after the vCJD disease was first reported in 1996, concerns were raised about the possibility of transmission between british humans through blood transfusion (Ponte, 2006). The risk was purely hypothetical in nature, as there was no evidence of transfusion transmission having taken place. The first probable case of transfusion-transmitted vCJD would not be identified until late 2003 , the second in 2004 . And yet, many nations implemented regulations aimed at reducing the risk of such transmission while the risk was still hypothetical in nature (Ponte, 2006). So the American scientist is skeptical about blood transfer, when both cases were again published in Lancet. The second case was even less conclusive because it is a case of preclinical vCJD in a patient who died from a non-neurological disorder 5 years after receiving a blood transfusion. There should still be a known a third case, on February 9, 2006, announced by the UK Health Protection Agency. However, it is unknown from literatury sources. So, like the panic of

transmitting BSE to humans, the panic of transmitting vCJD by blood transfusion, ended in both cases.

After the discovery of a new infectious vCJD disease, the scientists concerned began to interest if the disease could be transmitted by surgical instruments. Concerns that surgical instruments may transmit vCJD have been raised by the finding of PrPSc (scrapie prion protein) not only in nervous, but also in lymphatic tissue (1997-99; published mostly by Ironside JW, Collinge J, Hill AF). Instruments used for tonsillectomy or appendectomy on unrecognized vCJD sufferers could become contaminated with the agent. In 2001 some scientists „hypothetical“ found, that prions are readily and tightly bound to stainless steel surfaces and can transmit scrapie to recipient mice after short exposure times. This system mimics contaminated surgical instruments and will allow an assessment of sterilisation procedures. However, ten years later the same scientists (Weissmann, Collinge), in a starting new discovery, have shown for the first time that abnormal prions, that can cause fatal neurodegenerative disease, can suddenly erupt from healthy brain tissue (Edgenworth et al., 2010). Their study offers experimental proof that prions can in fact originate spontaneously, and shows that this event is promoted by contact with steel surfaces. Co-author of this study, Julie Edgenworth stated: "One theory for our observations is that the metal acts as a catalyst to promote the creation of prions from the normal prion protein present in brain tissue…"

NOTE; Ten years have been haunted by this "hypothetical" surgical transmission of infection, and even now, after almost another 10 years, this fear persists.

2001- 2006; Three experts strongly opposed BSE infection

Despite continued investigation the origin of BSE is not certain. BSE does not resemble any strain of scrapie. The whole idea of `strains' is based on prion disease in mice. Mice have no known naturally occurring prion disease.Therefore, the disease the mice get is not BSE and it is not variant CJD. Therefore, the temporal relationship between BSE and variant CJD only coincidentally supports the notion that BSE caused variant CJD, and as such is not strong evidence. The evidence other than this comes from research using mouse models and analysis of subtypes of abnormal prion protein. This supporting evidence is related to papers published in high-ranking journals (1996-1999). The temporal relationship between BSE and vCJD (1990s) only coincidentally supported the notion that BSE caused vCJD, and as such is not evidence. Indeed, BSE has just appeared spontaneously (Brown, 2001).

NOTEe; Professor David Ronald Brown, research scientist notable for his work on prion diseases. He served as a member of SEAC, the British government advisory board on BSE and related diseases.

In 1996 a new variant of CJD was described and tentatively linked to BSE as a possible cause. What was initially a speculation has now evolved into orthodoxy among the medical profession in the United Kingdom if not the whole of Europe. In this paper I examine the evidence for a causal link between new variant CJD and the BSE prion and argue in favour of the alternative hypotheses that the variant is not caused by the prion and is not new (Venters, 2001).

NOTE; Physician George A. Venters is a UK consultant in public health medicine.

Another German scientist Ronald Scholz, opposing BSE/ vCJD infection theory, says that there is no sound biochemical basis for believing the prion to be an infective agent which, if it entersthe digestive system, can cause damage to the brain. In his view, therefore, the alleged oral transmissibility, either within or across species, has no proper foundation. He points out that the procedure cited as demonstrating transmissibility, i.e. injecting material from the brain of diseased animals directly into the brain of another, is not a valid model of infection and certainly does not prove that the disease can cross between species. Moreover, he points out that no experiment of controlled feeding with MBM has been published. The author having examined the science related to prions concludes that there must be grave doubts about the hypothesis's validity (Scholz, 2006).

NOTE; Professor Roland Scholz, physician and biochemist, research and teaching in the field of metabolism biochemistry, including dietary and metabolic diseases, formerly active at the Institute for Physiological Chemistry, Physical Biochemistry and Cell Biology at the Ludwig-Maximilians-Universität Munich.

2006- 2012; Is prion protein really infectious or is merely a secondary marker of the presence of the scrapie agent?

To determine the mechanisms of intestinal transport of infection and early pathogenesis, of sheep scrapie, isolated gut-loops were inoculated (Jeffrey et al., 2006). While their published research confirms that under laboratory circumstances prion protein can be absorbed across the gut, it also shows that this is unlikely to occur in real life. In addition, the results show that the places in the gut that do take up these disease-associated proteins are different from the

locations where infectivity is known to be amplified. This research questions whether prion protein is really infectious, and it suggests that prion protein is merely a secondary marker of the presence of the scrapie agent. If that is so, as their findings indicate, it might also be so for ostensibly infectious nature of prions in vCJD. That, however, leaves open the issue of what the disease's infectious agent might be (Jeffrey et al., 2006).

Roger Highfield as science journalist in his report- article "Can this really kill you?"(The Telegraph; May 30, 2006) wrote; “The Nobel prize-winning hypothesis that infectious proteins can cause CJD and 'mad cow disease' is still being challenged…But even today, and almost a decade after Prusiner's Nobel prize, findings still challenge his hypothesis so that, at best, it seems incomplete and, at worst, it may even be wrong”.

Six years later, Prusiner´s team (Stohr et al., 2012) found the right recipe to show that the amyloid-beta (Abeta) protein involved in Alzheimer´s disease (AD) are prions. So really, abnormal proteins are a consequence of the disease process, rather than a cause? In the AD, the most prevalent cerebral proteopathy, the two principal aggregating proteins are β-amyloid (Aβ) and tau. Pusiner´s team investigators inoculated transgenic mice with purified brain-derived Aβ fibrils or aggregates made of synthetic Aβ peptides. Their results provide incontrovertible evidence that Aβ aggregates are prions and that the formation of Aβ prions does not require additional proteins or co-factors. This was the first to definitively show that Aβ deposition can be seeded in the brain by synthetic Abeta alone, solidifying the conclusion that a prion-like process of corruptive protein templating is involved. Knowing that amyloid-β and similar proteins act like prions, researchers are left wondering why no one has recorded a case of the proteins passing from person to person, when on the basis of laboratory results, all neurodegenerative diseases should be infectious…Taken together, these results point to amyloid-β and other neurodegenerative proteins as behaving like prions, says Neil R. Cashman, a neurologist at the University of British Columbia, in Vancouver. “It’s becoming a widely accepted idea,” he adds. “But it’s also opening a Pandora’s box” (Wolf, 2012).

However to date, Pandora’s box is still closed and the BSE still raises among people fears, that humans can infect from cows by tainted meat, infectious medical equipment, by infectious blood... As far as the economic consequences are concerned, these are enormous finances losses in the European Union, where all MBM produced since 2001 is disposed of (mostly incinerated) and american soya is being compensated for this loss of protein. It has been calculated that in Europe is annually destroyed 16 million tonnes of MBM, who is replaced by 23 million tonnes of soybeans. In 2010, it was considered to lift

the ban on the use of MBM in animal feed in EU countries, when there were enough reasons to lift this ban (Ziggers, 2010).

Unfortunately, the European Commission finally did not give its approval. However, in the United States, Canada , where BSE has also been detected, MBM continues to be used in pig and poultry. In addition, from 1989 the UK was exporting about 25,000 tonnes of MBM to EU countries and about 7,000 tonnes to nations outside Europe mostly in the Middle East and Africa. By 1991, sales of MBM to Europe dropped to zero. At the same time exports of MBM to the Third World had soared to 30,000 tonnes (Barnett, 2000). However, not to Japan (see after two decades later); in Japan 36 cases and in Third World, no case of BSE.

References

ALMOND,J.W. - BROWN,P. - GORE, S. M. - HOFMAN, P. W.- WIENTJENS, R.M.- RIDLEY, H.F.- BAKER, G..- ROBERTS, W.- TYLER, K.L. (1995); Creutzfeldt-Jakob disease and bovine spongiform encephalopathy: any connection? BMJ. 311 ; 1415–1421.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC2544410/pdf/bmj00620-0039.pdf

BARNETT, A. (2000): Feed banned in Britain dumped on Third World.The Guardian, 29 October 2000.
https://www.theguardian.com/uk/2000/oct/29/bse.focus

BROWN,D.R. (2001); BSE did not cause variant CJD: an alternative cause related to post-industrial environmental contamination. Med Hypotheses. 57(5); 555-560. https://www.ncbi.nlm.nih.gov/pubmed/11735310

DENNY,G.O.- WILESMITH,J.W.- CLEMENTS,R.A.- HUESTON,W.D. (1992); Bovine spongiform encephalopathy in Northern Ireland: epidemiological observations 1988-1990.Vet Rec. 130(6); 113-116. https://www.ncbi.nlm.nih.gov/pubmed/1561742

DEWHURST, R.J.- MOORBY, J.M.- DHANOA, M.S.- EVANS, R.T.- FISHER, W.J. (2000); 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(8); 1782- 1794. https://www.ncbi.nlm.nih.gov/pubmed/10984155

EDGEWORTH, J.A. - GROS,N.- ALDEN,J.- JOINER,S.- WALDSWORTH,J.D.E.- LINEHAN,J.- BRANDNER,S.- JACKSON, G.S.- WEISSMANN,Ch.- COLLINGE,J (2010); Spontaneous generation of mammalian prions. PNAS, 107(32); 14402–14406.
https://www.pnas.org/content/107/32/14402

HIGHFIELD, R. (2006); Can this really kill you?. The Telegraph, May 30, 2006. https://www.telegraph.co.uk/technology/3346022/Can-this-really-kill-you.html
LAMBERT,V. (2010); Does vCJD still pose a major public health threat? The Telegraph, February 8, 2010. https://www.telegraph.co.uk/news/health/news/7168326/Does-vCJD-still-pose-amajor-public-health-threat.html
LIEWELYN,C.A.- HEWITT, P.E.- KNIGHT, R.S.- AMAR, K- COUSENS, S.- MACKENZIE, J.- WILL,R.G. (2004); Possible transmission of variant CreutzfeldtJakob disease by blood transfusion. Lancet, 363; 417- 421. https://www.ncbi.nlm.nih.gov/pubmed/14962520
MOORBY,J.M.- DEWHURST,R.J.- TWEED,J.K.- DHANOA, M.S.- BECK, N.F. (2000); Effects of altering the energy and protein supply to dairy cows during the dry period. 2. Metabolic and hormonal responses. J Dairy Sci., 83(8); 1795-1805. https://www.ncbi.nlm.nih.gov/pubmed/10984156
MOORBY,J.M.- DHANOA, M.S.- AUSTIN,A.R. (2000a); Aspects of the metabolism of dairy cows during the incubation of bovine spongiform encephalopathy. Vet Rec., 147(15); 409-412. https://www.ncbi.nlm.nih.gov/pubmed/11072985
JEFFREY, M.- GONZALES, L.- ESPENES,A. - PRESS, C.M.- MARTIN, S.- CHAPLIN, M.- DAVIS, L.- LANDSVERK, T.- MacALDOWIE, C.- EATON, S.- McGOVERN, G. (2006); Transportation of protein across the intestinal mucosa of scrapie-susceptible and scrapie-resistant sheep. J Pathol., 209(1); 4-14. https://www.ncbi.nlm.nih.gov/pubmed/16575799
PONTE, M.L. (2006); Insights into the Management of Emerging Infections: Regulating Variant Creutzfeldt-Jakob Disease Transfusion Risk in the UK and the US. Plos Medicine, 3 (10) ; 1751- 1763. https://www.ncbi.nlm.nih.gov/pubmed/17076547
SCHOLZ, R. (2006/3); Phantom BSE-Gefahr. Irrwege von Wissenschaft und Politik im BSE-Skandal. UMWELT & GESUNDHEIT; 94-99. http://www.iug-umwelt-gesundheit.de/pdf/2006-3_schwerpunkt1_BSE%20Scholz.pdf
STOHR,J- WATTS,J.C.- MENSINGER,Z.L.- OEHLER, A.- GRILLO, S.K.- DeARMOND, S.J.- PRUSINER, S.B.- GILES, K. (2012); Purified and synthetic Alzheimer's amyloid beta (Aβ) prions. PNAS, 109(27); 11025- 11030. https://www.ncbi.nlm.nih.gov/pubmed/22711819
VENTERS,G.A. (2001); New variant Creutzfeldt-Jakob disease: the epidemic that never was. BMJ. 323; 858–861. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC1121394/

WILESMITH,J.W.- WELLS,G.A.- CRANWELL, M.P,- RYAN,J.B. (1988); Bovine spongiform encephalopathy: epidemiological studies. Vet Rec. 123(25); 638- 644. https://www.ncbi.nlm.nih.gov/pubmed/3218047
WILL,R.G.- IRONSIDE, R.G.- ZEIDLER,J.W. - COUSENS, S.N.- ESTIBEIRO, K.- ALPEROVITCH, A.- POSER, S.- POCCHIARI, M,- HOFMAN, A.- SMITH, P.G. (1996); A new variant of Creutzfeldt-Jakob disease in the UK. Lancet. 347; 921-925. https://www.ncbi.nlm.nih.gov/pubmed/8598754
WINTER, M, H.- ALDRIDGE,B.M.- SCOTT,P.R.- CLARKE, (1989); Occurrence of 14 cases of bovine spongiform encephalopathy in a closed dairy herd. Brit.Vet. J. 145 (2); 191-194. https://www.ncbi.nlm.nih.gov/pubmed/2713640)
WOLF, L.K. (2012); Alzheimer's Prion Connection. Chemical and Engineering News, 90 (27); 24-26. https://cen.acs.org/articles/90/i27/Alzheimers-PrionConnection.html%7CAlzheimer%E2%80%99s%20Prion%20Connection
ZIGGERS, D. (2010); Meat and bone meal back into feed. All About Feed, 12 January 2010. https://www.allaboutfeed.net/home/meat-and-bone-meal-back-into-feed/

HLÁSNÝ, J. (2020); Is magnesium deficiency the cause of neurodegeneration in animals and humans? 1st part; BSE „magnesium- ammonia" alternative theory. Výzkum v chovu skotu, 62 (1), pp. 36- 48

https://www.vuchs.cz/main/bulletin/vyzkum-v-chovu-skotu-1-2020/

The neurodegenerative diseases, occurred to a greater extent, only in ruminants (BSE- cattle; scrapie- sheep; chronic wasting disease- deer), because only in them magnesium is not absorbed in the intestine, but in the rumen. Epidemiological incidence of neurodegenerative diseases in a certain period, was detected only in cattle in the UK, as the bovine spongiform encephalopathy (BSE). From the literature it is known that in British cows at 1980s was a higher incidence of clinical and subclinical (chronic), hypomagnesaemia (hypo-Mg), and a new BSE disease was found.

During the 1980s, the occurrence of hypo-Mg in the UK cows was complicated by higher protein intake, high level of ammonia in the rumen, without adequate supply of magnesium to the cattle feed ration. So, of all the

animals, they are just ruminants in which the lack of magnesium with the simultaneous protein excess in the diet (in the rumen) concentrates to the magnesium deficiency in their tissues.

Identified in 1986, BSE rapidly spread to affect UK herds although the incidence was very limited within individual herds. The source was assumed (on epidemiological grounds), to be from commercial feed which contained meat and bone meal (MBM) as an animal protein. The BSE cases dramatically declined after 1993, it it was ca 5 years later, when the ban of MBM feeding in cattle came into force (1988), so establishing this „infectious“ theory without a reasonable doubt.

See later, some doubts from one of the leading scientists regarding the BSE / vCJD infectious theory

Journalist Magnus Linklater visited (Jun 2006) the National CJD Surveillance Unit in Ediburgh who was run by Dr Richard Knight, a clinical neurologist. He was not only responsible for the UK's state of knowledge on the various strains of CJD, he also co-ordinated research across Europe. Journalist says; the total number of deaths (vCJD) since 1995 is 156, and the yearly figure has been in steady decline since 2000 - when 28 people died. Whatever happened to the great epidemic? Why are the figures falling? With refreshing candour, Dr Knight 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 journalist 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? My approach is rigorously empirical, Dr Knght answers. I am wedded to the principle that any scientific fact is simply a truth until the next experiment proves it to be false. When I say that BSE is the cause of vCJD, I have to be open to the idea that it may be proved false. But there is little doubt that there is a relationship between the cattle disease and the human disease, and all the facts point towards BSE as being the cause of vCJD. I know of no good evidence for any alternative.

Journalist says; in 1997, Stanley Prusiner, professor of neurology at the University of California, was awarded a Nobel Prize for proposing that the cause of brain diseases such as CJD, scrapie in sheep and BSE in cattle was a new type of infectious agent - not a virus or a bacterium, but a protein. He called these proteins as prions. However, experiments with sheep, given food

heavily contaminated with abnormal prions, showed that the animals simply digested them, with very few of the prions surviving. It seems possible that another, unidentified agent might be responsible for the disease. Even more challenging is the suggestion that the abnormal prions might be the consequence of the disease rather than its cause. So could there be another cause of the disease? It is possible for the prion theory to be false, but the cattle disease still to be the cause of vCJD? Dr Knight answers; it is possible that there is another agent involved, but I do not know anyone who has come up with an alternative (Linklater, 2006).

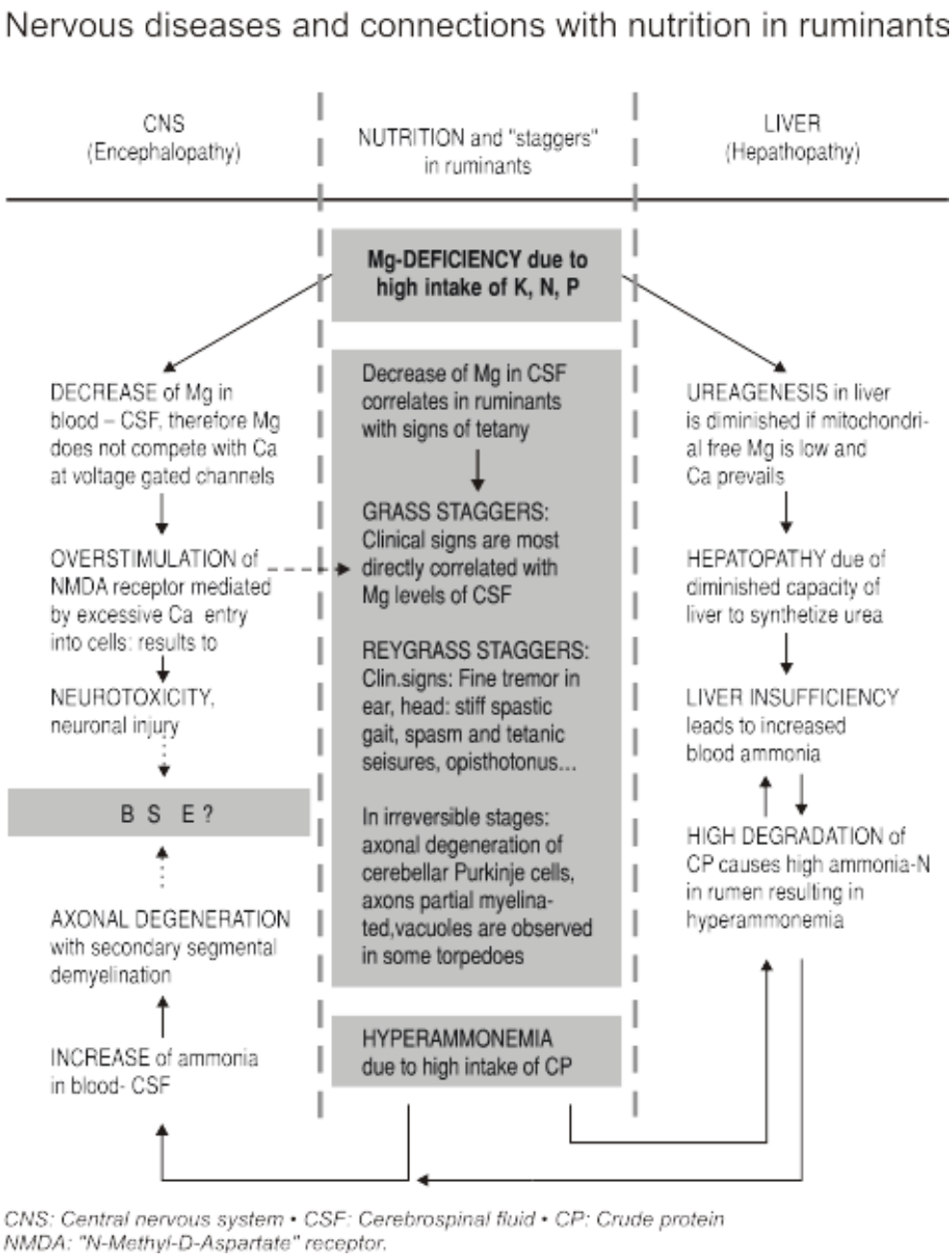
Recently we tried to show in the form of a critical review that in the first case (BSE) it was possible to rule out this infectious hypothesis as early as in 2006 and in the second case (vCJD) to exclude it completely in 2012. In this article it was stressed that there was no evidence of feeding MKM in cows (cattle) with the BSE occurence. On the contrary, there is evidence that BSE was found in cows without MKM feeding under normal and experimental conditions (Hlásný, 2019). So what could be another alternative regarding the cause of BSE? In 2006, Dr Knight says he doesn't know the other alternative? However, another alternative has been well known in Czech Republic since March 2001 as a "BSE magnesium- ammonia theory" (Hlásný, 2001), and internationally one year later (Hlásný, 2002).respo

Magnesium- ammonia theory as an alternative BSE hypothesis

This alternative „ magnesium- ammonia theory“ is based on the chronic Mg-deficiency potentiated by hyperammonemia in ruminants.There hypomagnesaemia plus hyperammonemia simultaneous action have a strong influence on the CNS, especially in ruminants (Mg absorption in the rumen), so that the BSE could has its roots in a more common nutritional problem. As a typical example about this; the ryegrass staggers is showed in ruminants. So, various clinical symptoms can be observed because the nervous system controlling both voluntary and unvoluntary muscles is affected (Mg and Ca disturbances) in the cattle females.

It seems, that during the chronic hypomagnesemic (hypo- Mg) disease, the heavy weather changes (cold- rainy, windy...) or nutrition (high intake of crude protein and potassium...) stress - these episodes of acute abruptions, may accelerate the nervous, like to the BSE disease symptoms. If the BSE is involved; a longer- chronic action of corresponding biochemical changes in the blood and cerebrospinal fluid (CSF) is necessary, to rise the irreversible neurodegenerative changes (Hlásný, 2001.

Fig 1



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

According to the Fig, 1 we have pointed out the following circumstances regarding the BSE alternative 'magnesium-ammonia theory'(Hlásný, 2001);

Mechanisms of action

Over the past 50 years, yield of many crops has been greatly increased, roughly in proportion to an increase in the use of the application of NPK fertilisers. Luxury consumption of potassium fertilisers leads to distortion of cation ratios

in the herbage: concentrations of Na, Mg and Ca are reduced relative to potassium. Higher N fertilisation increases N concentration in the herbage, appears to increase the concentrations of P and K, and decreases Mg utilisation by livestock causing hypo-Mg. Higher N concentration in forages gives higher fatty acid content and decreases the availability of feed Ca and Mg by the formation of insoluble Ca and Mg soaps in the gastrointestinal tract. Highly fertilised young herbage is characterised by a high crude protein content- this is highly degradable causing high rumen ammonia concentrations. At elevated blood concentrations, ammonia is toxic to the central nervous system (CNS).

Liver and hepatopathy

In the liver, excess of ammonium is generally considered to be eliminated via the ornithine cycle. Blood urea level is closely related to cyclic dietary protein intake, and is inversely proportional to the biological value of dietary protein. The high protein/ energy ratio also increases blood urea. The sum of the two processes does not result in accumulation of NH4 cations in the blood, but in hyperammonaemia (in ruminants, especially) because of the liver's diminished capacity to synthesise urea, and to a decrease in glutamine synthetase. In the fed state, most amino acids (except branched chain AA's) reaching the liver serve as precursors for ureagenesis in the ornithine cycle. Control of this cycle can be either long term, involving synthesis and degradation of enzyme molecules (hours or days), or short-term, via activation or inhibition of existing enzymes (seconds or minutes). Increases in enzyme activity are therefore observed after feeding a protein rich diet. Together, the two control systems allow large changes in flux through the ornithine cycle at a relatively constant ammonia concentration. Chronic and acute liver insufficiencies 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 since the clinical signs of chronic hepatic encephalopathy (HE) are reversible.

CNS and encephalopathy

In the CNS, the magnesium ion (Mg^{2+}) has two major functions: stabilising synaptic connections and enhancing neurochemical enzyme function. Mg^{2+} has been shown to increase the affinity of various binding proteins including the voltage-dependent calcium (Ca^{2+}) channel. Because Ca^{2+} ions are normally

low in most cells compared with extracellular fluid (ECF), only a small increase is needed, to significantly increase in the intracellular fluid. The most well characterised receptor for amino acid synaptic transmission is the N-Methyl-D-Aspartate (NMDA) receptor. Overstimulation of the NMDA receptor results in neurotoxicity and neural injury, involving the influx of Ca2+ into neurons. Studies have demonstrated that Mg can protect against NMDA-induced neurodegeneration, brain injury and convulsions.

Grass staggers and magnesium

Forages that cause staggers should be considered in the differential diagnosis of hypo-Mg. Expression of clinical signs is most directly correlated with cerebrospinal fluid (CSF) Mg levels. Commonly grass staggers (grass tetany) usually occurs in lactating cows during winter or spring when grasses are low in Mg, especially when energy intake is less than optimal and during adverse weather conditions. Affected cows may also have been pastured on rapidly growing forage crops that have been over-fertilised with potassium and nitrogen. In ryegrass staggers, affected animals have a characteristic stiff, spastic gait… recovery may be rapid if animals are not stressed (within 2 weeks after removal of the forage).

However, in the irreversible stages (longer pasture standing), axonal degeneration of cerebellar Purkinje cells has been found. Axonal changes (degeneration and vacuoles) are more likely to be found in cattle and sheep if the longer the clinical signs have been present. Epidemiological studies to date have shown BSE to occur mainly in countries where significantly higher amounts of NPK fertilisers are applied to forage crops. So above mentioned mechanisms should also be incorporated into the BSE research.

Why the BSE epidemic occured in the UK, especially ?

Rainfall and the available water capacity of the soil were major forage yield determinants, with output in the UK ranging from 6.000 to 14.000 kg dry matter (DM) /ha, under intensive fertilization in 1980s. There are notable exceptions such as Benelux, wchich although characterized by the highest pasture yields in Europe, however with a comparatively low share of grassland in total ruminant feed composition (50- 55%) compared with Ireland (97%), U.K. (83%), France (71%). In Britain perennial ryegrass is the most important species of sown

pastures, but Italian ryegrass (Lolium multiflorum), timothy (Phleum pratense), cocksfoot and the fescues (Festuca spp.) are also common (Lee, 1988).

*T*he composition of the 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 (McDonald et al., 1988). According to the „50 – year review“ about the fertilizer applications in the UK (Hemingway, 1999), there was mayby highest nitrogen fertilizers consumption in the world; in England and Wales, especially (1983-1988). This author´s summary indicates that in the UK there was the intention to use the high N-fertilization (and K-fertilization) for intensive silage production, especially. For example in 1983- 1988 period, in England and Wales; higher rates were used for intensive silage production; 201 kg (nitrogen), 15 kg (phosphorus) and 50 kg (potassium) per ha.

In contrast, recommended applications from MAFF (1994) were much higher; 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. Later, nitrogen application rates to grass have progressively declined. Present overall fertilizer use for grazing on dairy farms is about 170 kg N, 10 kg P and 20 kg K per ha (Hemingway, 1999).

In general, in the UK ; a high intake of grasses in ruminants; available water capacity, high N (and K-fertilization by animal excrements), cool and cold marine climatic region; these circumstances are ideal for the subclinical (chronic) hypo-Mg in ruminants. In addition, with the high probability after significant increase of crude protein in dairy cow rations (ARC, 1980) in mid-1980s, which was without equality of oral Mg supplementation.

Utilization of magnesium(Mg) in the ruminant gastrointestinal tract

Pastures in areas with intensive livestock production are generally rich in K due to frequent fertilization with manure, which may be injected directly into the soils (Schonewille et al., 1997). Grazing cattle on such pastures entails the risk of hypomagnesaemia (hypo-Mg), primarily due to a K-induced decrease in Mg absorption. When feeding legumes (clover), hypo-Mg is not detected in cows compared to grasses feeding (Hlásný, 1991). .

In ruminant animals, Mg absorption mainly takes place in the rumen (Tomas and Potter, 1976). Mg is absorbed by an active transport mechanism from the forestomachs (mainly the rumen) (Martens and Schweigel, 2000) and is influenced by a variety of factors with acute, transient or persistent effects. Acute rise of ruminal ammonia concentration decreases Mg absorption

transiently for 2–3 days (Gäbel and Martens, 1986). However, the nutrient having the greatest adverse effect on Mg absorption is an excess of K in the ration, as shown by sheep experiments (Grace et al., 1988; Dalley et al., 1997).The risk for hypo-Mg occurrence in lactating cows is increased with dietary characteristics of intensively managed forages, such as high concentrations in K, rapidly fermentable protein, and low concentrations in Mg. Increasing dietary K linearly decreases Mg absorbability in cows (Schonewille et al., 2008).

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. As this protein is readily fermentable, it leads to increased intraruminal ammonia concentrations up to 30- 70 mmol/l (Martens and Rayssiguier, 1980) and to a decrease in the availability and absorption of Mg (Martens and Schweigel, 2000; Urdaz et al., 2003).

The chemical gradient of soluble Mg between the lumen and cell content is also of importance. Solubility in the rumen predicts Mg absorption in cattle, see when MgO is used in many cases to ensure a sufficient Mg intake. The soluble Mg concentration depends on the rumen pH. At a higher pH values Mg solubility declines sharply as ruminal pH rises above 6.0 – 6.5, observed by Dalley et al. (1997), which may relate to the formation of insoluble Mg - ammonium phosphates. Adequate amounts of fermentable carbohydrates are important in maintaining serum Mg levels, since Mg solubility and the absorptive surface area of rumen papilla both improve with availability of short chain fatty acids and lowered rumen pH (Martens and Schweigel, 2000).

Lush pastures often high in nonprotein nitrogen are relatively low in readily fermentable carbohydrates. The ability of the ruminal microbes to incorporate the nonprotein nitrogen into microbial protein is exceeded and ammonia and ammonium ion build up in the rumen increasing ruminal pH. Grazing animals tend to have higher ruminal pH because the high content of nitrogen and potassium positively correlate, in ryegrass especially (Hlásný, 1990).

A high rate and extent of degradation of crude protein causing high concentrations of ammonia – N in rumen results in hyperammonemia, because of diminished capacity of liver to synthetise urea in ornithine cycle. Of prime importance in the control of carbamoyl- phosphate synthese activity in ornithine cycle; is the mitochondrial concentration of N-acetylglutamate, a compound that is indispensable for enzyme activity. In addition to the absolute concentration of mitochondrial N-acetylglutamate, the concentration of liver mitochondrial free Mg ion may be relevant, since binding N-acetylglutamate to

carbamoyl- phosphate synthase is dependent on this action (Meijer et al, 1985; Meijer et al, 1990).

Magnesium ions (Mg2+) as a physiological antagonist of the *N*-methyl-D-Aspartate (NMDA) receptors

Magnesium is the fourth most abundant mineral in the body and the second-most abundant cation following potassium in mammalian cells. One of the main neurological functions of the Mg2+ is due to Mg2+ interaction with the N-methyl-D-Aspartate receptors (NMDARs). They are glutamate- and glycine-gated ion channels that exhibit unique biophysical properties including highly voltage-dependent channel block by physiological concentrations (~1 mM) of Mg2+. At resting membrane potentials, external Mg2+ ions enter the NMDAR pore, but unlike the permeant Ca2+ ions, they bind tightly and prevent further ion permeation (Nowak et al., 1984;Mayer et al., 1984).

The Mg2+ ions are present at millimolar concentrations in the external milieu of neurons, while intracellular Mg2+ concentrations are in the micromolar range, resulting in a net inward driving force for Mg2+ ions at negative membrane potentials. A depolarization of sufficient amplitude and duration is required to dislodge and repel the Mg2+ ions from the pore, thereby allowing the flow of permeant ions.

Glutamic acid (Glutamate) as the major excitatory neurotransmitter in the mammalian CNS, acts postsynaptically at several receptor types named for their prototypic pharmacological agonist. In excess, glutamate triggers a process called excitotoxicity, causing neuronal damage and eventual cell death, particularly when NMDARs are activated. Glutamate receptors (GluRs) are divided into two families, ionotropic and metabotropic. Metabotropic GluRs are G protein-coupled receptors, while ionotropic GluRs are ion channels. There are three types of ionotropic GluRs, namely AMPA, kainate, and NMDARs. .AMPA and kainate receptors are predominantly permeable to Na+ and K+ ions, whereas NMDARs (NR1, NR2A–D and NR3A–B) are predominantly Ca2+ ion permeable (Kew and Kemp, 2005).

The Mg2+ ions regulates several ion channels. especially in neurons, extracellular Mg2+ contributes to the activity control of one of the glutamate receptors, NMDAR, which plays crucial roles in neuronal functions (Kumar, 2015; Iacobucci et al., 2017). Studies have demonstrated that Mg2+ can protect against NMDARs- induced neurodegeneration, brain injury, and convulsions. Mg2+ competes with Ca at voltagegated Ca channels both intracellularly, and

on the cell surface membrane, Mg2+ is capable of blocking NMDA receptors both intracellularly and extracellularly.

The permeation pathway of NMDA receptors has a property that sets them apart from other conventional ligand- gated receptors. At hyperpolarized membrane potentials more negative than about −70 mV, the concentration of Mg2+ in the extracellular fluid of the brain is sufficient to virtually abolish ion flux through NMDA receptor channels even in the presence of the coagonists glutamate and glycine (Nowak et al., 1984). So in neurons at the resting membrane potential (−70 mV), Mg2+ blocks the NMDAR. When the membrane potential is increased to −30 mV, Mg2+ block is relaxed, and the NMDAR is activated.

Energetically compromised neurons become depolarized (more positively charged) because in the absence of energy they cannot maintain ionic homeostasis; this depolarization relieves the normal Mg2+ block of NMDA receptor-coupled channels because the relatively positive charge in the cell repels positively-charged Mg2+ from the channel pore. Hence, during periods of ischemia and in many neurodegenerative diseases, excessive stimulation of glutamate receptors is thought to occur. These neurodegenerative diseases, including Alzheimer's disease , are caused by different mechanisms but may share a final common pathway to neuronal injury due to the overstimulation of glutamate receptors, especially of the NMDA subtype (Lipton and Rosenberg, 1994).

During ammonia intoxication, NMDA receptors are excessively stimulated, resulting in a larger influx of Ca^{2+} than usual into neurons. This would elicit a cascade of reactions and eventually lead to neuronal cell death. How does ammonia cause excessive activation of NMDA receptors? It has been shown that NH_4^+ induced depolarization in cultured rat cortical astrocytes.. This ammonia-induced depolarization could also take place in neuronal membranes and result in removal of Mg^{2+} that normally blocks the NMDA receptor channel, leading to excessive activation of the NMDA receptor (Felipo and Butterworth, 2002).

As the NMDA receptor is involved in excitatory neurotransmission, neuroplasticity, and neuroexcitotoxicity, it plays an important role in developmental plasticity (Nechifor, 2018), learning and memory (Miyashita , 2012). Animal studies (Lambuk et al., 2018) have shown neuronal protection from pre-treatment with Mg, making this mineral of intense interest for its potential neuroprotective role in humans.

The Mg2+ serve as a blockade to the calcium channel in the NMDARs , and must be removed for glutamatergic excitatory signaling to occur (Stroebel et

al., 2018). Low magnesium levels may theoretically potentiate glutamatergic neurotransmission, leading to a supportive environment for excitotoxicity, which can lead to oxidative stress and neuronal cell death (Castilho et al., 1999). The NMDARs localization can be synaptic, perisynaptic, extrasynaptic, or even presynaptic, with receptor activity at each location coupling to specific cellular events. Activation of presynaptic NMDARs activates pro-survival signaling, while extrasynaptic NMDARs activity mediates pro-death signaling . Thus, while NMDARs receptors mediate key physiological functions such as learning and memory under normal conditions, they also play a role in glutamate excitotoxicity. which is involved in many neurodegenerative conditions.

A high incidence of magnesium deficiency in cows in the United Kingdom ?

Magnesium (Mg) is a nutrient required for all animals, but it is especially critical for ruminants. A physiological deficiency of Mg results in hypomagnesemic tetany. Typically, only female ruminants are affected, and the disturbance usually occurs during the early stages of lactation. To evaluate the situation of the Mg- deficiency (1982-92), publications from the journal Veterinary Record were selected. Monitoring in England and Wales was carried out by Department of Veterinary Clinical Studies, University of Edinburgh. One survey in Northern Ireland was conducted by Veterinary Sciences Division, Belfast.

England and Wales

The national incidence of hypomagnesaemia (hypo-Mg) in dairy cows is available from the Dairy Herd Health and Productivity Service (DHHPS). Data has been collected monthly (1982) from 206 farms, with an average herd size of 110 cows, on disease treatments.

Carefully pre-planed blood testing of cows typical of specified lactation groups is carried out three or four times a year, there was a 1 per cent average incidence of clinical hypo-Mg. The highest incidence was in May, but cases occured in all months. The incidence of subclinical hypo-Mg which was defined a a cow having a serum Mg level below 0.78 mmol/litre, was as high as 7 per cent of milkers and 15 per cent of dry cows tested in some months. The number of blood samples looked per month varied from 200 to 2000 (Whitaker and Kelly, 1982).

There was a substantial increase in the numbers of cases of clinical hypo-Mg in dairy cows in spring 1984 when 1 per cent were affected in May 1984 and 0.9 per cent in June (250 herds, average size 130 cows). This was two and half times greater than the May average for the previous three years and five times greater than the June average. Interpreted as a similar percentage of the United Kingdom national herd , there probably were 64.000 cases of clinical hypo-Mg in this two month period of 1984 (Whitaker et al., 1985).

Through the agency of DHHPS , which monitors 30.000 cows (1992), it has become apparent that the incidence of clinical and subclinical hypo-Mg in dairy cows seems to be still unusually high. Farmers are reporting losses and a significant proportion of blood samples are showing low Mg levels (Whitaker et al.,1993).

Northern Ireland

513 dairy herds were sampled during the grazing season from March to November 1991, and 1266 suckler herds from March 1991 to February 1992. Serum blood Mg below 0.8 mmol/l was found in 28% of cows (McCoy et al., 1993).

So usually the veterinary survey (1982- 1992) has been; the subclinical hypo-Mg was found in about 7-15 % of tested cows. However, after 1993/94 period, there is some evidence about the increase of additional dietary Mg-supplementation in the UK dairy rations. For example, currently the incidence of subclinical hypoMg in British dairy cows is estimated to be 3-4 percent. However, attention is still paid to Mg-deficiency about the incidence of clinical forms of hypo-Mg (Kumssa et al., 2019).

Higher additional dietary Mg-supplementation in dairy cows as an European „phenomenon“ at the beginning of 1990s; and decrease of BSE incidence in the UK ?

An experiment from the former Czechoslovakia from the late 1980s was carried out, under practical conditions in a larger set of high-pregnant heifers and cows after the first calving. Testing 260 tonnes of a new mineral supplement in young breeding cattle, it was found that it was necessary to increase the Mg content by 180% in existing commercially produced mineral supplements (Hlásný, 1989). These results as a field experiences were also patented by the“Czechoslovak Patent Office“ in Prague (Hlásný, 1991).

The phenomenon about the „European great Mg interest“ it was also seen at the 3rd European Congress on Magnesium in Geneve. There we presented above mentioned results from Czechoslovakia (Hlásný and Steidl, 1990), with my cooworker neurologist professor Ladislav Steidl (Palacky University, Olomouc), a member of the editorial board (1975- 2000) of the international journal Magnesium Research. These recommendations that much more magnesium is necessary in dairy mineral supplements, really it was commonly realized in Europe, at the beginning of 1990s.

As mentioned with regard to the monitoring of hypo- Mg in the UK, in the period around 1990, two workplaces this carried out. For England and Wales it was a laboratory in Edinburgh (Whitaker et al.), and for Northern Ireland a laboratory in Belfast (McCoy et al.). The following recommendations regarding Mg supplementation to cows are also known from these two sites;

The Mg- deficiency among British cows has been known since the 1960s. In early 1980s , it was stated that in most circumstances there is no safe alternative to providing extra dietary Mg, with daily 30 g of available Mg per lactating cow being an average target. Because mostly were available only commercial Mg-blocks with very considerable variation in palatability, mostly very low Mg intake (Whitaker et al., 1985).

Since the early 1990s, it was Mg gradually implemented in concentrates. As stated by Whitaker et al. (1993) the most realiable method of Mg- deficiency prevention is by feeding Mg in the daily feed ration concentrate allowance. So to achieve the extra dietary requirement level 30 g of daily Mg-intake with certainity, it was recommended Mg to include in feed ration. Thus not leaving any option to the dairy cow, about the Mg lower intake. In lactating cows at pasture, more palatable high Mg-cobs were recommended to use.

In British practice, calcined magnesites (MgO) it was an important source of supplemental Mg. However, solubility of MgO sources varies greatly in practice when Mg sources with smaller particle size have a higher solubility. Thus, it is recommended to assess the quality of calcined magnesites before use inpractice. At this time the only choice is incorporating Mg in concentrates fed at milking. All other approaches have significant practical drawbacks and have been seen to fail (Whitaker et al., 1993).

The 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. The use of time-lapse video recording (equipment) provides an additional method of carrying out such quality control. 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 intake; 4.4 g, 58.3 g, 8.7 g, 49.2 g, 15.0 g Mg- at 1,2,3,4,5 types of Mg blocks, resp. This study showed that available commercial Mg-blocks; have considerable variation in palatability, Mg-content and Mg-intake of the hardened Mg rich blocks . 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, it was concluded by authors (McCOY et al.,1994).

Thus in the begining of 1990s there is the special interest to monitoring of intake Mg-blocks by cattle; so this also is evidence of higher interest of Mg-supplying in the UK cows. All these recommendations point to a change in the early 1990s, an increase in Mg supplementation to British cows, and a decrease of Mg deficiency. Therefore if we will put this „phenomenon“ into practice; significantly higher additional dietary Mg-supplementation - can be a cause about the BSE incidence decrease in the UK, after 1993/94 period.

Evidence that this change occurred is also the fact that the monitoring of hypo Mg ended in 1993 and further activities of the two mentioned departments on the Mg issue is known later around 2000 (Whitaker et al., 2000; McCoy, 2004). In both cases, however, it was not the focus of the publication on the occurrence of hypo- Mg.

Recommendations about lower protein intake in dairy cows (NRC, 2001); and later steady decrease of BSE incidence in European states with high producing dairy cows

At the beginning of 1980s, for more productive high yielding dairy cows more of protein was required. So two new European systems- the ARC (1980) system in the United Kingdom and the PDI grele system in France (Verite et al., 1979)- were official proposals within each country. Higher protein intake also according to the NRC (1989) was recommended. However, improvements in the research about nitrogen metabolism changed the view on the protein intake in high producing dairy cows, to the end of 1990s. The previous edition of NRC (1989) was replaced by a new edtition (NRC, 2001).

There are changes about the lowering of protein requirements; in early lactation especially. During early lactation (0-70 days postpartum) milk production increases rapidly, peaking at 4 to 6 weeks after calving. Protein dietary content is critical during early lactation; feed rations may need to contain 19% of more crude protein in the dry matter of dairy ration (Ensminger et al., 1990). However, for example, the same high crude protein (CP) level is

recommended in turkey- in animals with highest protein requirements from the all domestic animals (NRC,1994). In growing young turkeys (age; 11 to 14 weeks) there is the recommendation 19 percent of protein of the diet (90% dry matter). Almost the same situation is in monogastric young rapidly growing pigs (NRC, 1998)- average weight in range 15 kg (20.9% of CP) and 35 kg (18% of CP) of the diet (90% dry matter).

Almost the same high protein recommendations (18.8 % of dry matter) are from McCullough (1994) to dairy rations of high producing „supercows"- however, during the „all lactation". The similar conditions were recorded in a nutritional experiment (Moorby et al., 2000), when 13 per cent of experimental cows, the clinical signs of BSE developed ! There during first 12 wk of lactation the content of protein was ca 20%, and during next 11 wk 17.5% in the dry matter of feed ration.

However, according to the NRC (1989) this high protein level it was recommended only during first three weeks (0-21 days postpartum) after calving. So above mentioned recommendation were „overdosed" in dairy cow practice. The research during 1990s resulted to decrease of protein content in dairy cow rations (CP; early lactation, especially); see the comparison of the NRC (1989 and 2001);

Dairy cow: 600-680 kg body weight				
	Lactation			Early lactation
Milk yield (kg/day)	35	45	55	35
Degradable protein – "DP" (%):				
NRC,1989	9.7	10.4	10.4	9.7
NRC,2001	9.7	9.8	9.8	10.3
Undegradable protein- "UDP" (%):				
NRC,1989	5.7	6.0	6.3	7.2
NRC,2001	5.5	6.2	6.9	5.6
Crude protein – "CP"- (%):				
NRC,1989	16.0	17.0	17.5	**19.0**
NRC,2001	15.2	16.0	16.7	**15.9**

Therefore if we will put into practice ; the recommendation from the NRC (2001) about the significant decrease of crude protein in the early lactation; this "phenomenon" can be a cause about the BSE incidence decrease in the

western Europe especially, after 2001. There were higher producing dairy cows in 1990s, compared with the eastern Europe countries..

During the 1980s, the occurrence of hypo-Mg in the UK cows was complicated by higher protein intake- high level of ammonia In the rumen. A new system has been introduced (ARC, 1980) and the dietary intake crude protein divided into undegradable dietary protein (UDP) and degradable dietary protein (DDP) fractions. However, this ARC publication, as an official proposal for this country, grossly overstimated the requirement of cattle for the protein (Alderman, 1993).

So it is possible that this warning in the UK contributed to the fact that during the 1990s there was a decrease in protein intake to cows (before the release of NRC 2001), simultaneously with increased Mg supplementation. The result was a rapid decline in the incidence of BSE after 1993, in the UK. When on the other hand, the American publication (high CP intake- supercows) continued to be used in other European countries (McCullough, 1994).

Conclusion

Based on this interpretation (BSE/Mg), should be similarly preventive do about Alzheimer's disease (AD) ? Absorption and in the body higher Mg-utilization it is also very important in people. American researchers have found (Slutsky et al. 2010) that a new highly absorbable form of Mg called Mg-L-threonate concentrates more efficiently in the brain, rebuilds ruptured synapses, and restores the degraded neuronal connections observed in AD.

Further continuation (Výzkum v chovu skotu) of this article will be called; Is magnesium deficiency the cause of neurodegeneration in animals and humans? 2nd part; Alzheimer´s disease and BSE magnesium- ammonia theory connections.

References

ALDERMAN,G. (1993); Energy and protein requirements of ruminants : an advisory manual prepared by the AFRC, 175 p.
https://www.cabdirect.org/cabdirect/abstract/19941400396

ARC 1980; The nutrient requirements of ruminant livestock. Commonwealth Agricultural Bureaux, UK, 351 p.
https://www.cabdirect.org/cabdirect/abstract/19812609956

CASTIHO, R.F.- WARD, M.W.- NICHOLLS,D.G. (1999); Oxidative stress, mitochondrial function, and acute glutamate excitotoxicity in cultured cerebellar granule cells. J Neurochem. ,72(4), pp. 1394-1401.
https://pubmed.ncbi.nlm.nih.gov/10098841/

DALLEY, D. - ISHERWOOD, P.- SYKES, A.- ROBSON, A. (1997); Effect of in vitro manipulation of pH on magnesium solubility in ruminal and caecal digesta of sheep. J Agric Sci., 129, pp. 107–111.
https://www.cambridge.org/core/journals/journal-of-agricultural-science/article/abs/effect-of-in-vitro-manipulation-of-ph-on-magnesium-solubility-in-ruminal-and-caecal-digesta-in-sheep/A10CA12A447946F5378A6AB0F4EB2FE7
ENSMINGER, M.E.- OLDFIELD, J.E.- HEINEMANN,W.W. (1990): Feeds and Nutrition; Clovis- Calif., 1544 p.
FELIPO, V.- BUTTERWORTH, R.F. (2002); Neurobiology of ammonia.
Prog Neurobiol., 67(4), pp. 259- 279. https://pubmed.ncbi.nlm.nih.gov/12207972/
GABEL, G.- MARTENS, H. (1986); The effect of ammonia on magnesium metabolism in sheep. Zeitschrift für Tierphysiologie, Tierernährung und Futtermittelkunde, 55, pp. 278–287.
https://agris.fao.org/agris-search/search.do?recordID=DE86A0385
Br J Nutr., 59, pp. 93-108GRACE, N.D.- CAPLE, I.W.- CARE, A.D. (1988); Studies in sheep on the absorption of magnesium from a low molecular weight fraction of the reticulo-rumen contents. https://pubmed.ncbi.nlm.nih.gov/3345310/
HEMINGWAY, R.G. (1999): 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. Animal Science, 69, pp. 1-18
https://www.cambridge.org/core/journals/animal-science/article/abs/effect-of-changing-patterns-of-fertilizer-applications-on-the-major-mineral-composition-of-herbage-in-relation-to-the-requirements-of-cattle-a-50year-review/F1E98F4DAFB39DE9424FF1B05C53EA0E
HLÁSNÝ, J. (1989); Evaluation of a new mineral feed supplement used in feeding young breeding cattle in the winter season. Vet Med (Praha), 34(12), pp. 717-725
https://www.ncbi.nlm.nih.gov/pubmed/2631375
HLÁSNÝ, J. (1990); Evaluation of some relationships between macrominerals and nitrogen compounds in forages. Agrochémia (Bratislava), 30 (1), pp. 28-32
HLÁSNÝ, J.- STEIDL, L. (1990); Mechanism of the origin of magnesium deficiency in feedstuffs and in nutrients. Magnesium Research, 3, p. 48
HLASNY, J. (1991); Mineral supplement for breeding cattle. Praha, Czechoslovak patent No. 274 171.
HLÁSNÝ, J. (1991): Influence of nutrition on acid base balance and serum magnesium in dairy cows. Biopharm (Praha), 1, pp. 51-60
HLÁSNÝ, J. (2001): Nervous diseases and connections with nutrition in ruminants. Výzkum v chovu skotu (Rapotín), 43 (1), pp.13-29
http://www.bse-expert.cz/pdf/BSE_publikace.pdf
HLÁSNÝ, J. (2002): Lone voices in the BSE debate. Feed Mix (International Journal on Feed, Nutrition and Technology- Elsevier Business Informations), 10 (2), p. 12-15
https://www.allaboutfeed.net/home/lone-voices-in-the-bse-debate/

HLÁSNÝ, J.(2019); Infectious diseases derived from meat and bone meal (MBM); epidemics that never was? Vyzkum v chovu skotu (Rapotin), 61 (2), pp. 23-30
IACOBUCCI, G.J. -POPESCU, G.K. (2017); NMDA receptors: linking physiological output to biophysical operation. Nat. Rev. Neurosci. 18, pp. 236–249. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5640446/
KEW, J.N.- KEMP, J.A. (2005); Ionotropic and metabotropic glutamate receptor structure and pharmacology. Psychopharmacology (Berl) ,179 (1), pp. 4-29. https://pubmed.ncbi.nlm.nih.gov/15731895/
KUMAR, A. (2015); NMDA Receptor Function During Senescence: Implication on Cognitive Performance. Front. Neurosci. 9, p. 473. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4679982/
KUMSSA, D.B.- , PENROSE, B. -, BONE, P.A. - L0VATT, J.A. - BRPADLEY, M.R. - KENDALL, N.R.- ANDER, E.L. (2019); A reconnaissance survey of farmers' awareness of hypomagnesaemic tetany in UK cattle and sheep farms. PloS One, 14,(10); e0223868. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6788701/
LEE,J. (1988): Forages. Livestock Production Science, 19, pp. 13-46 https://www.sciencedirect.com/science/article/abs/pii/0301622688900851
LINKLATER, M. (2006); BSE and the great scare of nvCJD. Spectrum Magazine of Scotland on Sunday, 25th June 2006
LIPTON, S.A.- ROSENBERG, P.A. (1994); Excitatory amino acids as a final common pathway for neurologic disorders. N Engl J Med. , 330(9), pp. 613- 622. https://www.nejm.org/doi/10.1056/NEJM199403033300907?url_ver=Z39.88-2003&rfr_id=ori:rid:crossref.org&rfr_dat=cr_pub%20%200pubmed
MARTENS, H. - SCHWEIGEL, M. (2000); Pathophysiology of grass tetany and others hypomagnesemias.Vet Clin North Am Food Anim Pract., 16, pp. 339–368. https://pubmed.ncbi.nlm.nih.gov/11022344/
MARTENS, H. - RAYSSIGUIER, Y. (1980); Magnesium metabolism and hypomagnesaemia. In Digestive Physiology and Metabolism in Ruminants, pp. 447-466 https://link.springer.com/content/pdf/bfm%3A978-94-011-8067-2%2F1.pdf
MAYER, M.L. --, WESTBROOK, G.L. - GUTHRIE, P.B.(1984); Voltage-dependent block by Mg of NMDA responses in spinal cord neurones. Nature, 309, p. 261. https://pubmed.ncbi.nlm.nih.gov/6325946/
McCOY, M.A.- GOODALL, E. A. - KENNEDY, D.G. (1993); Incidence of hypomagnesaemia in dairy and suckler cows in Northern Ireland. Vet Rec. ,132 (21), p. 537. https://pubmed.ncbi.nlm.nih.gov/8322445/
McCOY, M.A.- RICE, D.A.- WRIGHT, A.- KENNEDY, D.G (1994).: Use of time-lapse video equipment to determine the efficasy of commercial magnesium blocks in cattle. Vet Rec., 135, pp. 209-210
McCOY (2004); Hypomagnesaemia and new data on vitreous humour magnesium concentration as a post-mortem marker in ruminants. Magnes Res.17(2), pp.137- 145. https://pubmed.ncbi.nlm.nih.gov/15319147/

McCULLOUGH, M.E. (1994):. Total mixed rations and supercows.Hoard´s Dairyman, 63 p.
McDONALD, P.- EDWARDS, R.A.- GREENHALGH, J.F.D. (1988).:Animal Nutrition (4th edition), London, 543 p.
MEIJER, A.J.- LOF,C.- RAMOS,I.C.- VERHOEVEN,A.J. (1985); Control of ureagenesis. Eur J Biochem, 148, pp. 189-196 https://febs.onlinelibrary.wiley.com/doi/10.1111/j.1432-1033.1985.tb08824.x
MEIJER, A.J.- LAMERS,W.H.- CHAMULEAU,R.A. (1990); Nitrogen metabolism and ornithine cycle function. Physiol Rev, 70, pp. 701- 748 https://pubmed.ncbi.nlm.nih.gov/2194222/
MIYASHITA, T. - ODA, Y. - HORIUCHI, J. - YIN, J.C.P.- MORIMOTO, T.- SAITOE, M. (2012); Mg2+ block of Drosophila NMDA receptors is required for long-term memory formation and CREB-dependent gene expression. Neuron , 74, pp. 887–89 https://pubmed.ncbi.nlm.nih.gov/22681692/
MOORBY, J.M.- DEWHURST, R.J.- TWEED, J.K.S.- DHANOA, M.S.(2000): Aspects of the metabolism of dairy cows during the incubation period of bovine spongiform encephalopathy. Vet.Rec., 147, pp. 409-412
https://pubmed.ncbi.nlm.nih.gov/11072985/
NECHIFOR, M. (2018); Magnesium in addiction - a general view. Magnes. Res. , 31, pp. 90–98. https://pubmed.ncbi.nlm.nih.gov/30714574/
NOWAK, L. - BREGESTOVSKI, P.- ASCHER, P.- HERBET, A. - PROCHIANTZ, A. (1984); Magnesium gates glutamate-activated channels in mouse central neurones. Nature. 307, p..462. https://www.nature.com/articles/307462a0
NRC (1989); National Research Council. Nutrient Requirements of Dairy Cattle. 6th rev. ed. Natl. Acad. Sci., Washington, D.C.
NRC (2001); National Research Council. Nutrient Requirements of Dairy Cattle. 7th rev. ed. Natl. Acad. Sci., Washington, D.C.
SCHONEWILLE, J.T. - EVERTS, H. - JITTAKHOT, S.- BEYNEN, A.C. (2008);. Quantitative prediction of magnesium absorption in dairy cows. J Dairy Sci., 91, pp. 271–278. https://pubmed.ncbi.nlm.nih.gov/18096949/
SLUTSKY, A.N.- WU, L.J.- HUANG, C.- ZHANG, L.-LI, B.- ZHAO, X.- GOVINDARAJAN, A.- ZHAO, M.G.- ZHUO, M.-TONEGAWA, S.- LIU, G. (2010); Enhancement of learning and memory by elevating brain magnesium. Neuron, 65 (2), pp. 165- 177 https://www.ncbi.nlm.nih.gov/pubmed/20152124
STROEBEL, D.- CASADO, M.- PAOLETTI, P.(2018); Triheteromeric NMDA receptors: from structure to synaptic physiology. Curr Opin Physiol., 2, pp. 1-2 https://pubmed.ncbi.nlm.nih.gov/29682629/
TOMAS, F.M.- POTTER, B.J. (1976); The site of magnesium absorption from the ruminant stomach. Br J Nutr, 36, pp. 37–45.
https://pubmed.ncbi.nlm.nih.gov/949467/

URDAZ, J. -SANTOS, J. - JARDON, P. - OVERTON, M. (2003) ; Importance of appropriate amounts of magnesium in rations for dairy cows. JAVMA, 222, pp. 1518–1523 https://pubmed.ncbi.nlm.nih.gov/12784955/
WHITAKER, D.A.- KELLY, J.M. (1982); Incidence of clinical and subclinical hypomagnesaemia in dairy cows in England and Wales. Vet Rec. ,110 (19), pp. 450-451. https://pubmed.ncbi.nlm.nih.gov/7101698/
WHITAKER,D.A.- KELLY, J.M. - SMITH, E.J. (1985); Hypomagnesaemia increase. Vet Rec. ,116 (16), pp. 451- 452.
https://pubmed.ncbi.nlm.nih.gov/4002580/
WHITAKER, D.A.- KELLY, J.M. -EAYERS, H.F. (1993); Incidence of hypomagnesaemia. Vet Rec. ,132(26), p. 662.
https://pubmed.ncbi.nlm.nih.gov/8362475/
WHITAKER,D.A.- KELLY, J.M.- SMITH, S.(2000); Disposal and disease rates in 340 British dairy herds.Vet Rec. ,146(13), pp. 363-367
https://pubmed.ncbi.nlm.nih.gov/10803980/

HLÁSNÝ, J. (2020); Is magnesium deficiency the cause of neurodegeneration in animals and humans?2nd part; Alzheimer's disease and BSE magnesium-ammonia theory connections.Výzkum v chovu skotu, 62 (2) , pp, 28- 40

https://www.vuchs.cz/main/bulletin/vyzkum-v-chovu-skotu-2-2020/

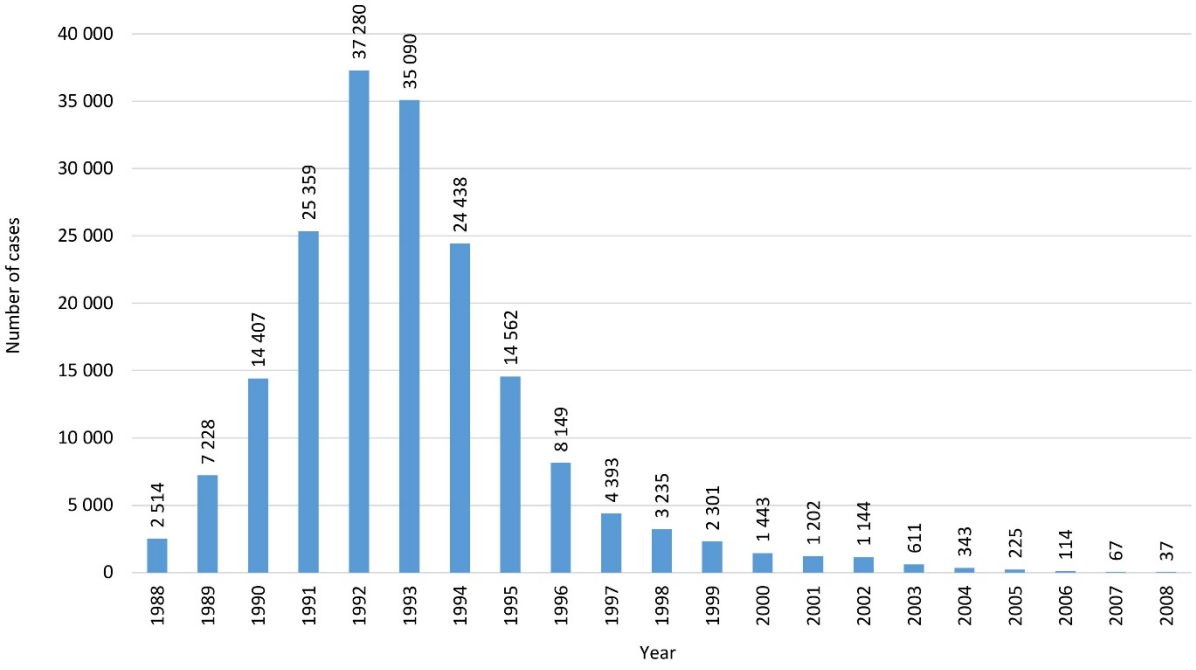
The aim of this study is to present an alternative nutritional hypothesis about the cause of bovine spongiform encephalopathy (BSE) in the United Kingdom (UK), based on a significant magnesium (Mg) deficiency in British cows. This could also contribute to elucidating the causes of Alzheimer's disease in humans.

Recently we tried to show another alternative „nutritional BSE theory“, based on the BSE occurrence in the UK, well known in Czech Republic for almost 20 years (Hlásný, 2001) as a "BSE magnesium- ammonia theory" (Hlásný, 2020). There is not an infection, but the basis is an ecological problem, where the result is a Mg deficit in the plant and in feed rations and thus also in animal tissue. This occurs especially in ruminants, in which mainly forages are used in the feed ration, when unlike other animals, Mg is not absorbed in the intestine, but in the rumen.

In this context, it should be recalled that during the 1980s there was a significant increase in the incidence of Mg deficiency in cattle in the UK. There the occurrence of hypomagnesaemia (hypo-Mg) in cows was complicated by higher protein intake, high level of ammonia in the rumen, without adequate supply of Mg to the cattle feed ration. However, of all the animals, they are just ruminants in which the lack of Mg with the simultaneous protein excess in the diet (in the rumen) concentrates to the Mg deficiency in their tissues. In addition, it seems that there is the similarity about the individual susceptibility in the hypomagnesaemic „indicator cows“ and the BSE, because the incidence of BSE within a dairy herd is very low- from 0.14 to 3.3% (Wilesmith et al., 1988).

FIG 1

Chronology of epidemic of bovine spongiform encephalopathy in United Kingdom, 1988-2008 (OIE, 2017)



Identified in 1986, the BSE rapidly spread to affect UK cattle herds although the incidence was very limited within individual herds. The source was hypothetically assumed, to be from commercial feed which contained meat and bone meal (MBM) as an animal protein. The BSE cases dramatically declined after 1993 (see Figure 1), it was ca 5 years later, when the ban of MBM feeding in cattle came into force (1988), so establishing this as an „infectious“ theory (incubation period about 5 years). However, there was no evidence of feeding

MBM in British cattle! When later (1996) researchers found that BSE is transmissible to humans (variant CJD), their infectious hypothesis was further strengthened, without any doubts. Other critical views on the origin of BSE have not been scientifically accepted , supported by American prion scientist, Stanley Prusiner, who won the Nobel Prize in 1997, about this subject. Thus, he cemented the reputation of BSE infectious theory oponents, as bad actors.

However, when the sudden occurrence, epidemic course and abrupt attenuation of the BSE epidemic it was supposed to be a challenge about more thorough scientific research! Especially when nothing like this has never happened in terms of the epidemic of a some new neurodegenerative disease in animals and humans.

Magnesium deficiency in cattle as an ecological – fertilization problem

Over the past about 70 years, yield of many crops has been greatly increased, roughly in proportion to an increase in the use of the application of NPK fertilisers. Luxury consumption of K fertilisers leads to distortion of cation ratios in the herbage: concentrations of Na, Mg and Ca are reduced relative to potassium. Higher N fertilisation increases N concentration in the herbage, appears to increase the concentrations of K and P, and decreases Mg utilisation by livestock, causing hypomagnesaemia (hypo-Mg).

Potassium (K) fertilizer application to increase forage production may contribute to an increased K/(Ca+Mg) ratio in forage plants, not only by adding K to soil, but also by displacing soil-adsorbed Ca and Mg by ion exchange, contributing to increased susceptibility of Ca and Mg to leaching loss from the root zone during rainy seasons. High K intake relative to Ca and Mg intake may induce hypo- Mg.

This „tetany ratio“ is expressed as K / (Ca+Mg) in milliequivalents (mEq) per kg of dry matter. Milliequivalents take into account both molecular weight and valence (i.e. charge) because acid-base balance is affected by electrical charge rather than mass. The K/(Ca+Mg) ratio exceeding 2.2 in forages has been commonly considered a risk factor for grass tetany (Kemp and t´Hart, 1957). Generally in ruminants, high K intake results in decreased absorption of Mg from the digestive tract.(Wylie et al, 1985; Schonewille et al, 1999).

a/ Some findings (Pisek district) from the late of 1980s

In the former Czechoslovakia, the consumption of potassium fertilizers increased significantly during the 1970 - 1980s, so that the ratio between N fertilizers and K fertilizers consumed was very narrow at the end of the 1980s , in terms of world scale (Table 1). At that time, during the 1980s, an extensive laboratory examination of feed was carried out in South Bohemia (Pisek District) in order to find out what change in feed occurred at a higher intensity of fertilization. See the published results (Hlásný, 1989b, Hlásný, 1989c). Even before these two scientific publications were published, the largest (to date) "ecological seminar", in response to excessive potassium fertilization, took place in South Bohemia (Písek), on February 14-15, 1989, with the participation of more than 600 active experts from the all Czechoslovakia. (Hlásný, 1989a; Honz and Hlásný, 1989).

Computer evaluation of the nutrients and macroelements content from 750 samples of fodder crops in 50 different stages and the order of mowing found that by comparison with tabular values published 15 to 20 years ago, the potassium (K) content in feed dry matter (except maize) increased significantly. For example, content of K in the clover was found to be 31 relative percent higher, the calcium (Ca) and magnesium (Mg) by 41% lower, and the sodium (Na) content by six to seven times lower. Higher sugar concentrations (reducing sugars after inversion) were clearly found in fodder from the first mowings, although the K content was higher than in other mowings. This trend was most pronounced in clover. The value of the calcium to phosphorus ratio is higher in those feeds in which lower values of the K / (Ca + Mg) ratio are commonly found and vice versa. The value of this ratio is lowest in alfalfa (1.5 to 1.7), followed by clover (1.7 to 2.0), grasses such as Italian ryegrass (4 to 8), cereal forages such as oats, wheat, rye (5 to 8). Higher values of K / (Ca + Mg) in the reported range were found in the younger vegetation phase. From the point of view of prevention of cattle metabolic disorders, it is appropriate to evaluate fodder crops according to sugar content and K / (Ca + Mg) values (Hlásný, 1989b).

NOTE; The values of the K / (Ca + Mg) ratio in the feeds were evaluated as the ratio of the content of these elements in the feeds; the reason being the simplification about the computer processing of a large set of laboratory results.

By means of computer evaluation of the results of 1280 analyzed samples, representing 238 671 tons of dry matter (corn silage- 41 %, clover-grass silage - 39 %, hay grass – 20%) from preserved feedstuffs (South Bohemia; potato

production region) it was found that they contained 2.2% potassium (K), 0.52% calcium (Ca), 0.19% magnesium (Mg), 0.28% phosphorus (P) and 0.031% sodium (Na). These results differ significantly from the results of fodder analyzes taken 15 - 20 years ago; e.g. the dry matter of clover-grass material presently contains 56% more K and conversely 64% less Na, 36% less Mg and 33% less Ca. Similar tendencies have been found in the other preserved feedstuffs. This general finding is inconsistent with the present prescription of mineral mixtures, designated for the winter feeding period in dairy cows with a daily intake of about 250 g K (Hlásný, 1989c).

A set of 53 metabolic tests was compiled, in the examination from 793 cows, in a detailed assessment - calculation of the feed ration, from the 1978-1988 period. Cows were fed forage with K/ (Ca+ Mg) ratio ranged from 2 to 6. It was found that amount of K does not directly influenced magnesaemia and acid-base balance. It is important to know both K/(Ca +Mg) and carbohydrate/ crude protein ratio in the feed ration to predict metabolic disturbances. The relation of these two ratios was always in significant negative correlation wit the value of urine acid- base and pH. Presumably, K/(Ca + Mg) value in the forage equal or exceeding 3,0 it could predict an onset of hypo- Mg (Hlásný, 1991).

b/ Consumption of fertilizers in the critical "BSE period", around 1990

The results presented above were obtained (Pisek district) in the area where the consumption of industrial fertilizers averaged 253 kg NPK / ha, which represented the average of the former Czechoslovakia.

Table 1. Consumption of NPK fertilizers in some countries, comparing data from 1990 and 1995 (FAO, 1996)

		Nitrogen	P2O5	K2O	N : P : K
		millions of tons			
Czechoslovakia	1990	704820	437000	460600	1 : 0,62 : 0,65
Czech Republic	1995	254000	46500	40020	1 : 0,18 : 0,16
Slovak Republic	1995	80250	16623	13800	
		47%	14%	12%	
Netherlands	1990	412356	76095	98318	1 : 0,18 : 0,24
	1995	380000	64000	73000	1 : 0,17 : 0,19
		92%	84%	74%	
United Kingdom	1990	1582000	428000	525000	1 : 0,27 : 0,33
	1995	1412000	421000	465000	1 : 0,30 : 0,33
		93%	98%	89%	
Italy	1990	827279	607927	377713	1 : 0,73 : 0,46
	1995	879200	584700	427000	1 : 0,67 : 0,49
		106%	96%	113%	
USA	1990	10047935	3941442	4719847	1 : 0,39 : 0,47
	1995	10632059	4006651	4647210	1 : 0,38 : 0,44
		106%	102%	98%	

A similar and somewhat lower consumption of NPK fertilizers (approximately 240 kg / ha) was around 1990 in the UK (AIC, 2019), with a significantly more favorable NPK nutrient ratio, in terms of K fertilization intensity and the occurrence of Mg deficiency in cattle (Table 1). However, with some exceptions, no acute clinical form of hypomagnesaemia was found in the Pisek district, however, the proportion of grasses on agricultural land was at the level of about 20%, with a higher proportion of clover on arable land. On the other hand in the UK, comparatively high share of grassland in total ruminant feed composition (83%) and well known is high protein content in young, heavily. fertilized grasses (rainfall and the available water capacity...).

According to the Agricultural Industries Confederation (AIC, 2019), fertiliser usage in Great Britain has reduced significantly over the last 30 years (1983- 2017). Through greater understanding and improving farming methods fertiliser use has decreased by over 30% for nitrogen, 55% for phosphate and 45% for potash. There stably highest consumption of fertilizers was in the period from 1983 to 1991, after about 1997 there was a steadily declining consumption of fertilizers. In Czech Republic the consumption of fertilizers signifficantly decreased from 1990 to 1995, K and P, especially (Table 1). And on the contrary, especially N consumption increased, from 1995 to the present (personal communication).

Alzheimer´s disease and magnesium deficiency

According to own findings (Pisek district), it was confirmed the well known knowledge that luxury consumption of K fertilisers leads to distortion of cation ratios in the herbage: concentrations of Na, Mg and Ca are reduced relative to potassium, see high consumption of K fertilizers with N ; P; K ratio at the level 1; 0.62; 0.65. The result was a reduction in the Mg content in forages by about 40%, compared to the situation 15-20 years ago. An even higher reduction was found for Na, which, however, can be easily compensated by NaCl supplementation in cattle nutrition, as well as a reduction in Ca content by the limestone supplementation.

Alzheimer´s disease (AD) is the most common dementia type and may account for 60–70% of dementia cases. The incidence of AD has positive correlation with age, and more female patients are afflicted with AD than male. AD progression is associated with the selective loss of neurons in the hippocampus and neocortex, brain areas involved in memory and cognition. Hippocampal neurons are primarily affected due to the vulnerability of pyramidal cell synapses at the early stage of AD, which eventually leads to functional impairment in cognition and memory. Presently, the drugs available for AD treatment, including cholinesterase inhibitors and an antagonist of the N-methyl-D-aspartate receptor (memantine), can only inhibit dementia symptoms for a limited period of time but cannot stop or reverse disease progression. Effective or disease-modifying drugs for AD are still lacking. (Huang et al., 2020).

a/ The Mediterranean diet rich in magnesium is considered as a prevention of Alzheimer's disease

Researchers are looking at whether a healthy diet also can help preserve cognitive function or reduce the risk of Alzheimer’s. The Mediterranean diet (MeDi) is one factor that was initially shown (N Engl J Med., 2003) to reduce of the risk of mild cognitive impairment (MCI) and dementia. MeDi is characterized by a high intake of vegetables, legumes, fruits, cereals, and unsaturated fatty acid...Higher adherence to the MeDi was associated with reduced risk of MCI and AD. The subjects in the highest MeDi tertile had 33% less risk of MCI (Balwinder et al., 2014). However, recent studies have found no significant association between dietary patterns, including Mediterranean diet, and risk for dementia (Akbaraly et al., 2019), indicating that adherence to

healthy dietary patterns may not be enough to reduce the risk to develop age-related cognitive impairment and dementia.

Why is the MeDI no longer so effective in preventing AD?

Green leafy vegetables, such as spinach, legumes, nuts, seeds, and whole grains, are good Mg sources. In general, foods containing dietary fiber (food of plant origin) provide the most Mg. The Mediterranean diet is characterized by a high intake of vegetables and legumes, which should be richest in Mg content from all foods, but the question arises whether, as with feedstuffs after years of intensive NPK fertilization, there has also been a decrease in Mg content?

b/ Alzheimer´s disease (AD); magnesium and N-Methyl-D-Aspartate (NMDA) receptor relationships

Magnesium (Mg) is the second most abundant cation in mammalian cells, and it is essential for numerous cellular processes including enzymatic reactions, ion channel functions, metabolic cycles, cellular signaling, and DNA/RNA stabilities. Because of the versatile and universal nature of Mg^{2+}, the homeostasis of intracellular Mg^{2+} is physiologically linked to growth, proliferation, differentiation, energy metabolism, and death of cells. On the cellular and tissue levels, maintaining Mg^{2+} within optimal levels according to the biological context, such as cell types, developmental stages, extracellular environments, and pathophysiological conditions, is crucial for development, normal functions, and diseases.

Hence, Mg^{2+} is pathologically involved in cancers, diabetes, and neurodegenerative diseases, such as Parkinson's disease, Alzheimer's disease, and demyelination. In the research field regarding the roles and mechanisms of Mg^{2+} regulation, numerous controversies caused by its versatility and complexity still exist. As Mg^{2+}, at least, plays critical roles in neuronal development, healthy normal functions, and diseases, appropriate Mg^{2+} supplementation exhibits neurotrophic effects in a majority of cases. Hence, the control of Mg^{2+} homeostasis can be a candidate for therapeutic targets in neuronal diseases (Yamanaka et al., 2019). Mg is essential for regulation of muscle contraction (including that of the heart), blood pressure, insulin metabolism, and is required for the synthesis of DNA, RNA, and proteins. In the nervous system, Mg is important for optimal nerve transmission and neuromuscular coordination, as well as serving to protect against excitotoxicity (Grober et al., 2015).

One of the main neurological functions of Mg is due to Mg's interaction with the N-Methyl-D-Aspartate (NMDA) receptor. Mg serves as a blockade to the Ca channel in the NMDA receptor, and must be removed for glutamatergic excitatory signaling to occur. Abnormal glutamatergic neurotransmission has been implicated in many neurological and psychiatric disorders including: migraine, chronic pain, epilepsy, Alzheimer's, Parkinson's, and stroke, in addition to depression and anxiety, which are commonly comorbid with these neurological disorders (Olloquequi et al., 2018) .

The role of Mg in degenerative diseases has been the focus of increased attention in recent years. Continuous low Mg intake for two generations induces exclusive loss of dopaminergic neurons in rats (Oyanagi, 2005), and may support the Mg hypothesis in the pathogenesis of parkinsonism- dementia complex. Mg supplementation prevents the loss of dopaminergic neurons and ameliorates neurite pathology in a PD model, indicating a role of Mg in protection of dopaminergic neurons in the substantia nigra from degeneration (Hashimoto *et al.*, 2008).

New findings in animal studies are promising and provide novel insights into the neuroprotective effects of Mg, and Mg treatment at the early stage may decrease the risk of cognitive decline in AD. Mg deficiency has been emerging as a risk factor for AD. The level of Mg diet is critical to maintain synaptic plasticity, and the decline in hippocampal synaptic connections has been associated with impaired memory (Fan et al., 2017). Physiological concentrations of Mg are essential for synaptic conduction, and required for normal functioning of the nervous system. It has various effects at different concentrations on intellectual and neuronal functions via many bio-chemical mechanisms, including NMDA-receptor responses to excitatory amino acids and calcium influx (Nowak *et al.*, 1984; Mayer *et al.*, 1984), inhibition of calcium channels (Iseri and French, 1984) and glutamate release (Lin *et al.*, 2002), effects on cell membrane fluidity and stability (Ebel and Gunther, 1980), and toxic effects of calcium (Alvarez-Leefmans *et al.*, 1987). These mechanisms published mostly in 1980s, have important roles in chronic neuronal degeneration and subsequent development of dementia.

Magnesium defict was found in many brain regions of patients with AD when compared with age-matched controls (Andrasi et al., 2005). Decreased Mg level was found in various tissues of AD patients in clinical and laboratory studies (Barbagallo et al., 2011). The Mg^{2+} of cerebrospinal fluid (CSF) is greater than that of blood , indicating that Mg^{2+} is actively transported from the blood into CSF. The alteration of Mg^{2+} of CSF correlates with the extracellular Mg^{2+} around neurons, which affects neural activities. Thus, the Mg^{2+} of CSF

is closely related to various brain functions (Wong et al., 2009). In particular, the Mg2+ of CSF and cognitive functions have been reported to exhibit a positive correlation (Sun et al., 2016). In addition, the intracellular Mg2+ of erythrocytes significantly correlates with the Mg2+ of CSF in the hippocampus, and further with the hippocampal synapse density and recognition and memory performance (Xiong et al., 2019), suggesting that Mg2+ of erythrocytes is a good index of recognition and memory. Even under severely Mg2+-depleted conditions, up to 80% of dietary Mg2+ can be absorbed (Graham et al., 1960), and most of Mg2+ in whole body exchanges at a very slow rate with biological half-time of 1000 hours (Avioli and Berman, 1966). Even in such conditions, the Mg2+ in the serum is maintained within the normal range (De Baaij et al., 2015).

Extracellular fluid (ECF) in the central nervous system (CNS) is separated from the blood circulation by the blood–brain barrier (BBB). The BBB comprises endothelial cells of brain capillaries and allows passage of nutrients and electrolytes for the maintenance of ECF homeostasis. Because neuronal and glial cells are closely located with a distance of 20 to 50 nm and the volume of extracellular space is quite small in brain unlike the other organs (Rusakov and Kullmann, 1998), concentrations of the ECF components is greatly fluctuating. Thus, the BBB actively transport several molecules for the ECF homeostasis. The Mg2+ in ECF is maintained within a greater level compared with that of plasma or CSF (Bito et al., 1969), the gap provides evidences for the active transport of Mg2+ in BBB.

Generally, the activation of synaptic NMDA receptors (NMDARs) initiates plasticity and stimulates cell survival. In contrast, the activation of extrasynaptic NMDARs promotes cell death and thus contributes to the etiology of AD, which can be blocked by an AD drug, memantine, an NMDAR antagonist that selectively blocks the function of extrasynaptic NMDARs (Wang and Reddy, 2017).

In addition, there can be another example about hypoglutamatergic condition when also cannabinoids (like Mg) can inhibit progression of certain neurodegenerative diseases. The endocannabinoid system is widespread throughout the central nervous system and its type 1 receptor (CB1) plays a crucial role in preventing the neurotoxicity caused by activation of NMDA receptors . Indeed, it is the activity of NMDA receptors themselves that provides the demands on the endogenous cannabinoids in order to control their calcium currents. Therefore, a physiological role of this system is to maintain NMDA receptor activity within safe limits, thereby protecting neural cells from

excitotoxicity (Sanchez- Blazquez et al., 2013). So there is evidence to support the hypothesis that the cannabinoid system can limit the neurodegenerative possesses that drive progressive disease, and may provide a new avenue for disease control. Cannabis use can be a proof about the link between the NMDA receptor hyperfunction- neurodegeneration and hypofunction -schizophrenia (Hlásný, 2008).

c/ A new magnesium supplement and Alzheimer's disease treatment

In 2004, researchers at Massachusetts Institute of Technology (MIT) demonstrated that Mg2+ helps regulate synapse strength by modulating NMDA receptors (Slutsky et al., 2004). Later, a second study from the same group found that aged rats that were given a new Mg supplement had improved memory and learning ability. Researchers discovered and patented magnesium L-threonate (MgT) based on its unique ability to boost the blood–brain barrier (BBB) levels of Mg (Slutsky et al., 2010). Interestingly, the amount of Mg2+ in the brain increased by only ~15% during the chronic MgT treatment. Nonetheless, this small increase in brain Mg2+ concentration was sufficient to reduce the memory deficits in aged rats. Rapid absorption and ability to enter the brain enables this Mg to structurally reverse certain aspects of brain aging.

Research has shown that once MgT gets into the brain, it increases the density of synapses, which are the communication connections between brain cells. This is critical because loss of synaptic density is associated with brain shrinkage and cognitive decline (Fox et al.,1996). The most startling finding is a reversal of more than ten years in clinical measures of brain aging in people who supplemented with MgT . Recent animal and human studies demonstrates the benefits of MgT also in adult humans with cognitive dysfunction, sleep disorders, and anxiety (Huang et al., 2018; Shen et al., 2019; Liu et al. 2016).

Although there are numerous basic evidences showing that Mg can inhibit pathological processes involved in neuroglial degeneration, this low-cost option is not well-considered in clinical research and practice for now. Nevertheless, none of the expensive drugs currently recommended by the classic guidelines (in addition to physiological rehabilitation) had shown exceptional effectiveness. Herein, focusing on AD, we analyze the therapeutic pathways that support the use of Mg for neurogenesis and neuroprotection. According to experimental findings reviewed, Mg shows interesting abilities to facilitate toxin clearance, reduce neuroinflammation, inhibit the pathologic processing of

amyloid protein precursor (APP) as well as the abnormal tau protein phosphorylation, and to reverse the deregulation of NMDA receptors (Toffa et al., 2019).

d/ Alzheimer's disease (AD); dysfunction of NMDA receptors and amyloid- beta accumulation

The AD is characterized by progressive cognitive impairment and distinct neuropathological lesions in the brain, including intracellular neurofibrillary tangles, and extracellular, parenchymal and cerebrovascular senile plaques (Braak and Braak, 1991). Senile plaques are constituted of a 39–42 amino acid peptide, amyloid-β protein, which is generally accepted as being neurotoxic and playing a central role in the pathogenesis of neuronal dysfunction and synaptic failure in AD (Selkoe, 1991).

In the light of Mg as an antagonist of the NMDA receptor, chronic NMDA receptor activation decreased α-secretase-mediated amyloid protein precursor (APP) processing and increased amyloid-β (Aβ) production in cultured cortical neurons (Lesne et al., 2005). Furthermore, several lines of evidence suggest that APP metabolism and Aβ levels are closely correlated with neural activity in animals and humans (Buckner et al., 2005). It has been demonstrated that decreasing neuronal activity by high Mg (10 mM MgCl2) resulted in significant reduction of Aβ secretion, which may involve a change in APP processing (Kamenetz et al., 2003).

It is widely believed that soluble oligomeric forms of Aβ perturb synaptic function and plasticity. Long-term potentiation (LTP) is impaired while long-term depression (LTD) is facilitated by Aβ . Studies indicate that Aβ-induced alterations in synaptic function and plasticity require the activation of GluN2B-NMDA receptors , as GluN2B antagonists rescued Aβ-induced impairment of LTP , Aβ-induced loss of synapses and synaptic proteins (Rönicke et al., 2011). Further evidence suggests that Aβ affects predominantly the extrasynaptic NMDA receptors (Li et al., 2011), which are largely GluN2B-containing.

It was found that Aβ modulated NMDA -induced responses and vice versa; pre-exposure to Aβ decreased NMDA -evoked Ca2+ rise and pre-exposure to NMDA decreased Aβ response. In addition, simultaneous exposure to Aβ plus NMDA synergistically increased Ca2+ levels, an effect mediated by GluN2B-containing NMDA receptors (Ferreira et al., 2012). The NMDA receptors dysregulation evoked by Aβ and the consequent loss of Ca2+ homeostasis are

thought to be related to the early cognitive deficits observed in AD. Many researches have demonstrated that Aβ oligomers and NMDA receptor contribute to the synaptic dysfunction in AD (Li et al., 2016). Early neuronal dysfunction induced by Aβ is mediated by an activation of NR2B-containing NMDA receptor in primary neuronal cultures and hippocampal slices from rat and mouse. Since GluN2A subunits have been implicated in protective pathways, whereas GluN2B subunits appear to increase neuronal vulnerability. Activation of GluN2A and decrease in GluN2B subunit may be an attempt to reduce Aβ induced neuronal dysfunction (Liu et al., 2007).

Although amyloid plaques are regarded as a pathological hallmark of AD, the causal relationship between amyloid deposition and neurodegeneration was unclear for a long time. Aβ has widespread distribution through the brain and body, even in cognitively normal individuals. Soluble Aβ exerts a physiological function, modulating synaptic function and facilitating neuronal growth; furthermore, Aβ protects the brain from infections, repairs leaks in the blood–brain barrier, and promotes recovery from injury (Puzzo et al. 2015).

However, therapeutic administration of Mg is still controversial regarding the treatment of AD, and high doses of Mg may have potential detrimental side effects (Kieboom et al., 2017). The predominance of parasympathetic dysfunction in mild cognitive impairment (MCI) suggests that neurodegeneration may be due to an early cholinergic deficiency that involves central autonomic network in dementia (Collins et al., 2012).

From the overall point of view, all the "magnesium - NMDA receptor" relationships listed above have already been presented at four World Veterinary Congresses (2008-2017). Unfortunately, without any practical response from the domestic or global veterinary public (Hlásný.2008; Hlásný, 2013a; Hlásný, 2013b; Hlásný, 2015; Hlásný, 2017a; Hlásný, 2017b).

Conclusions

Neurodegenerative diseases occur to a greater extent only in ruminants (BSE-cattle; scrapie- sheep; chronic wasting disease- deer), because only in them the magnesium (Mg) is not absorbed in the intestine but in the rumen. The epidemiological incidence of neurodegenerative diseases at some time was found only in bovine animals in the United Kingdom (UK) as the BSE.

In the CNS, the magnesium ion (Mg^{2+}) has two major functions: stabilising synaptic connections and enhancing neurochemical enzyme function. The most well characterised receptor for amino acid synaptic transmission is the N-Methyl-D-Aspartate (NMDA) receptor. Overstimulation of the NMDA receptor

results in neurotoxicity and neural injury, Studies have demonstrated that Mg2+ (as a physiological- natural NMDA receptor antagonist) can protect against NMDA-induced neurodegeneration, brain injury and convulsions. If the lower the Mg2+ level in the animal tissue cells, the more marked is Ca2+ effect excitotoxicity (neurodegeneration). Then after significantly higher Mg-supplementation should be the incidence of BSE significantly reduced.

Based on this interpretation (BSE/Mg), as alternative "BSE magnesium-ammonia theory“, should be similarly preventive about Alzheimer's disease (AD). Researchers have found that a new highly absorbable form of Mg called Mg-L-threonate concentrates more efficiently in the brain, rebuilds ruptured synapses, and restores the degraded neuronal connections observed in AD.

However, Mg as a prevention of AD is still not generally presented in the scientific literature, the cause may be the effect of Mg on the autonomic nervous system.

Further continuation (Výzkum v chovu skotu) of this article will be called; Is magnesium deficiency the cause of neurodegeneration in animals and humans? 3rd part; Alzheimer´s disease and magnesium - parasympathetic dysfunction connections.

References

AIC (2019); *Agricultural Industries Confederation.* UK fertiliser consumption trends and statistics. Fertiliser use in Great Britain. https://www.agindustries.org.uk/resource/fertiliser-consumption-in-the-uk.html

AKBARALY.T.N.- SINGH-MANOUX, A.- DUGRAVOT, A.- BRUNNER, E.J.- KIVIMAKi, M. - SABIA, S. (2019); Association of Midlife Diet With Subsequent Risk for Dementia. *JAMA*, 321(10), pp. 957- 968, file:///C:/Users/hlasny/Downloads/jama_akbaraly_2019_oi_190018.pdf

ALVAREZ-LEEFMANS, F.J.- GIRALDEZ, F. - GAMINO, S.M. (1987); Intracellular free magnesium in excitable cells: its measurement and its biologic significance. *Can J Physiol Pharmacol*., 65, pp. 915–25, https://pubmed.ncbi.nlm.nih.gov/3113706/

ANDRASI, E. - PALI, N.- MOLNAR, Z. - KOSEL, S. (2005); Brain aluminum, magnesium and phosphorus contents of control and Alzheimer-diseased patients. *J Alzheimers Dis*. 7, pp. 273-284, https://pubmed.ncbi.nlm.nih.gov/16131728/

AVIOLI, L.V. - BERMAN, M. (1966); Mg28 kinetics in man. *J. Appl. Physiol*., 21, pp.1688– 94, https://pubmed.ncbi.nlm.nih.gov/5929290/694

BALWINDER, S.- PARSAIK, A.K.- MIELKE, M.M. et al. (2014); Association of Mediterranean diet with mild cognitive impairment and Alzheimer´s disease; A systematic review and meta- analysis, *J.Alzheimers Dis.*, 39 (2), pp. 271- 282, https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3946820/

BITO, L.Z. (1969); Blood-Brain Barrier: Evidence for active cation transport between blood and the extraceliular fluid of brain. *Science*. 165, pp.81–83,https://www.science.org/doi/10.1126/science.165.3888.81

BUCKNER, R. L.- SNYDER, A.Z.- SHANNON, B.J. et al. (2005); Molecular, structural, and functional characterization of Alzheimer's disease: evidence for a relationship between default activity, amyloid, and memory. *J Neurosci*., 25, pp. 7709–17, https://pubmed.ncbi.nlm.nih.gov/16120771/

COLLINS, O.- DILLON,S.- FINUCANE, C.- LAWLOR, B.- KENNY, R.A. (2012); Parasympathetic autonomic dysfunction is common in mild cognitive impair-ment. *Neurobiol Aging*, 33, pp.2324-2333, https://pubmed.ncbi.nlm.nih.gov/22188719/

De BAAIJ, J.H.F. - HOENDEROP, J.G.J. - BINDELS, R.J.M. (2015); Magnesium in man: implications for health and disease. *Physiol. Rev*., 95, pp. 1–46, https://pubmed.ncbi.nlm.nih.gov/25540137/

EBEL, H.- GUNTHER, T. (1980); Magnesium metabolism: a review. *J Clin Chem Clin Biochem.*,18, pp. 257–70, https://www.degruyter.com/document/doi/10.1515/cclm.1980.18.5.257/html

FAN, X, - WHEATLEY, E.G.- VILLEDA, S. A. (2017); Mechanisms of Hippocampal Aging and the Potential for Rejuvenation. *Annu Rev Neurosci*, 40, pp. 251–72, https://pubmed.ncbi.nlm.nih.gov/28441118/

FAO (1996); *Fertilizer* (Rome), 45, p. 146.

FERREIRA, I. L. - BAJOUCO, L. M. - MOTA, S. I. - AUBERSON, Y. P. - OLIVEIRA, C. R. - REGO, A. C. (2012); Amyloid beta peptide 1–42 disturbs intracellular calcium homeostasis through activation of GluN2B-containing N-methyl-D-aspartate receptors in cortical cultures. *Cell Calcium*, 51, pp. 95–106, https://pubmed.ncbi.nlm.nih.gov/22177709/

GRAHAM, L.A., - CAESAR, J.J. - BURGEN, A.S. (1960); Gastrointestinal absorption and excretion of Mg 28 in man. *Metabolism*. 9, pp. 646–659, https://pubmed.ncbi.nlm.nih.gov/13848379/

GROBER, U. - SCHMIDT, J. - KISTERS, K. (2015); Magnesium in prevention and therapy. *Nutrients.* 7, pp. 8199–8226, https://pubmed.ncbi.nlm.nih.gov/26404370/

HASHIMOTO, T.- NISHI, K.- NAGASAO, J.- TSUJI, S.- OYANAGI, K. (2008); Magnesium exerts both preventive and ameliorating effects in an in vitro rat Parkinson disease model involving 1-methyl-4-phenylpyridinium (MPP+) toxicity in dopaminergic neurons. *Brain Res.*,1197, pp.143–51, https://pubmed.ncbi.nlm.nih.gov/18242592/

HIGHFIELD, R. (2006); Can this really kill you?. *The Telegraph,* May 30, 2006, https://www.telegraph.co.uk/technology/3346022/Can-this-really-kill-you.html

HLÁSNÝ,J. (1989a): Společným jmenovatelem draslík. *Náš chov* (Praha), 48, pp. 238-239.

HLÁSNÝ,J. (1989b): Content of macrominerals, nitrates, and soluble sugars in feedstuffs. *Vet Med (Praha*), 34 (9), pp. 567-576.

HLÁSNÝ,J. (1989c): Mineral mixtures and the macroelement composition of preserved roughage. *Vet Med (Praha)*, 34 (11), pp. 689- 697.

HLÁSNÝ, J. (1991): Influence of nutrition on acid base balance and serum magnesium in dairy cows. *Biopharm (Praha*), 1, pp. 51-60.

HLÁSNÝ, J. (2001): Nervous diseases and connections with nutrition in ruminants. *Výzkum v chovu skotu* (Rapotín), 43, (1), pp. 13-29, http://www.bse-expert.cz/pdf/BSE_publikace.pdf

HLÁSNÝ, J. (2008); Neurodegenerative diseases and schizophrenia as a hyper or hypofunction of the NMDA receptors. *29th World Veterinary Congress* (Canada, Vancouver),

HLÁSNÝ, J. (2013a); Hypotheses and facts about BSE/vCJD in the UK, so meat and bone meal as infectious agens should be revised. *31st World Veterinary Congress* (Czech Republic, Prague),

HLÁSNÝ, J. (2013b); The origins of the neurodegenerative diseases may lie in chronic magnesium deficiency coupled with a high protein intake. *31st World Veterinary Congress* (Czech Republic, Prague),

HLÁSNÝ,J. (2015); Alzheimer´s disease and bovine spongiform encephalopathy (BSE) connections. *32nd World Veterinary Congress* (Turkey, Istanbul).https://www.researchgate.net/publication/322699386_Alzheimers_disease_and_bovine_spongiform_encephalopathy_BSE_connections_32nd_WVC_in_Istanbul_2015_Poster_PP-_O60

HLÁSNÝ,J. (2017a); Causes and origin of the bovine spongiform encephalopathy (BSE) incidence in the United Kingdom? *33rd World Veterinary Congress* (South Korea, Incheon),
file:///C:/Users/hlasny/Documents/204.KOREA%20Za%C5%99az.posteru%20do%20EPIDEMIOLOGY.pdf

HLÁSNÝ,J. (2017b); Bovine spongiform encephalopathy (BSE) prevention and Alzheimer´s disease (AD) Connections? *33rd World Veterinary Congress* (South Korea, Incheon),
file:///C:/Users/hlasny/Documents/205.KOREA%20za%C5%99az.posteru%20do%20Bovine%20Medicine.pdf

HLÁSNÝ, J. (2020); Is magnesium deficiency the cause of neurodegeneration in animals and humans? 1st part; BSE „magnesium- ammonia“ alternative theory. *Výzkum v chovu skotu* (Rapotín), 62, pp. 36- 47. https://www.vuchs.cz/main/bulletin/vyzkum-v-chovu-skotu-1-2020/

HONZ,J.-HLÁSNÝ,J. (1989): Půda-hnojení-pícniny-skot. *Veterinářství* (Brno), 38, pp. 282-283.

HUANG, Y.- HUANG, X.- ZHANG, L. et al. (2018); Magnesium boosts the memory restorative effect of environmental enrichment in Alzheimer's disease mice. *CNS Neurosci Ther*., 24(1), pp.70–79, https://pubmed.ncbi.nlm.nih.gov/29125684/
HUANG,L.K.- CHAO,S.P.- HU, C.J. (2020); Clinical trials of new drugs for Alzheimer disease. *J Biomed Sci*., 27(1), pp.18 – 26, https://pubmed.ncbi.nlm.nih.gov/31906949/
ISERI, L.T.- FRENCH, J.H. (1984); Magnesium: nature's physiologic calcium blocker. *Am Heart J*.,108, pp.188–193, https://pubmed.ncbi.nlm.nih.gov/6375330/
KAMENETZ, F.- TOMITA, T.- HSIEH, H. et al. (2003); APP processing and synaptic function. *Neuron*, 37, pp. 925–37, https://pubmed.ncbi.nlm.nih.gov/12670422/
KEMP,A.- t´HART,M.L. (1957);Grass tetany in grazing milking cows.Archives, 5 (1), https://library.wur.nl/ojs/index.php/njas/article/view/17745
KIEBOOM, R.- LICHER, S.- WOLTERS, F.J. et al. (2017); Serum magnesium is associated with the risk of dementia. *Ne*urology, 89(16), pp.1716-1722, https://pubmed.ncbi.nlm.nih.gov/28931641/
LESNE, S. - ALI, C. - GABRIEL, C. et al, (2005); NMDA receptor activation inhibits alpha-secretase and promotes neuronal amyloid-beta production. *J Neurosci*.,25, pp.9367–77, https://pubmed.ncbi.nlm.nih.gov/16221845/
LI, S.- JIN, M.- T. KOEGLSPERGER, T.- N.E. SHEPARDSON, N. E.- G.M. SHANKAR, G.M.- SELKOE, D.J. (2011); Soluble Aβ oligomers inhibit long-term potentiation through a mechanism involving excessive activation of extrasynaptic NR2B-containing NMDA receptors. *J. Neurosci*., 31 (18), pp. 6627-6638, https://pubmed.ncbi.nlm.nih.gov/21543591/
LI, Y. - CHANG, L. - SONG, Y. - GAO, X. - ROSELLI, F. - LIU, J. et al. (2016); Astrocytic GluN2A and GluN2B oppose the synaptotoxic effects of amyloid-β1-40 in hippocampal cells. *J. Alzheimers Dis*., 54, pp. 135–148, https://pubmed.ncbi.nlm.nih.gov/27497478/
LIN, J.Y. - CHUNG, S.Y. - LIN, M.C.- CHENG, F. C. (2002); Effects of magnesium sulfate on energy metabolites and glutamate in the cortex during focal cerebral ischemia and reperfusion in the gerbil monitored by a dual- probe microdialysis technique. *Life Sci.*, 71, pp. 803–11, https://pubmed.ncbi.nlm.nih.gov/12074939/
LINKLATER, M. (2019); BSE prophecies of doom need a second look. *The Times.* April 1, 2019.
LIU, G. - WEINGER, J.G.- LU, Z.L. et al. (2016); Efficacy and Safety of MMFS-01, a Synapse Density Enhancer, for Treating Cognitive Impairment in Older Adults: A Randomized, Double-Blind, Placebo-Controlled Trial. *J Alzheimers Dis.*, 49 (4), pp. 971-90, https://pubmed.ncbi.nlm.nih.gov/26519439/
LIU, Y. -WONG, T.P. - AARTS, M. Et al. (2007); NMDA receptor subunits have differential roles in mediating excitotoxic neuronal death both in vitro and in vivo. *J Neurosci*., 27(11), pp.2846–2857, https://pubmed.ncbi.nlm.nih.gov/17360906

OIE (2017); Number of cases of bovine spongiform encephalopathy (BSE) reported in the United Kingdom. https://www.oie.int/en/disease/bovine-spongiform-encephalopathy/

OLLOQUEQI, J. - CORNEJO- CORDOVA, E. - VERDAGUER, E. et al, (2018); Excitotoxicity in the pathogenesis of neurological and psychiatric disorders: Therapeutic implications. *J. Psychopharmacol*., 32, pp. 265–275, https://pubmed.ncbi.nlm.nih.gov/29444621/

OYANAGI, K.(2005); The nature of the parkinsonism- dementia complex and amyotrophic lateral sclerosis of Guam and magnesium deficiency. *Parkinsonism and Related Disorders*,11, pp.17–23, https://pubmed.ncbi.nlm.nih.gov/15885623/

PUZZO,D.- GULISANO,W,- ARANCIO,O. - PALMERI, A.(2015); The keystone of Alzheimer pathogenesis might be sought in Abeta physiology. *Neuroscience*. 307, pp. 26–36, https://pubmed.ncbi.nlm.nih.gov/26314631/

RONICKE, R.- OONICKE, M.- MIKHAYLOVA, M. et al. (2011);Early neuronal dysfunction by amyloid β oligomers depends on activation of NR2B-containing NMDA receptors. *Neurobiol. Aging*, 32 (12), pp. 2219-2228, https://pubmed.ncbi.nlm.nih.gov/20133015/

RUSAKOV, D.A. - KULLMANN, D.M. (1998); Extrasynaptic glutamate diffusion in the hippocampus: ultrastructural constraints, uptake, and receptor activation. *J. Neurosci*., 18, pp.3158–3170, https://www.jneurosci.org/content/18/9/3158

SANCHEZ- BLAZQUEZ, P.- RODRIGUEZ-MUNOZ,M.- GARZON, J. (2013); The cannabinoid receptor 1 associates with NMDA receptors to produce glutamatergic hypofunction: implications in psychosis and schizophrenia. *Front Pharmacol*., 4, 169, doi; 10.3389. https://pubmed.ncbi.nlm.nih.gov/24427139/

SCHONEWILLE, J.T.- Van't KLOOSTER, A.T.- H. WOUTERSE, H.- BEYNEN, A.C. (1999); Effects of intrinsic potassium in artificially dried grass and supplemental potassium bicarbonate on apparent magnesium absorption in dry cows. *J. Dairy Sci*., 82, pp. 1824–1830, https://www.journalofdairyscience.org/article/S0022-0302(99)75413-5/pdf

SELKOE, D. J. (1991); The molecular pathology of Alzheimer's disease. *Neuron*., 6, pp. 487–98, https://pubmed.ncbi.nlm.nih.gov/1673054/

SHEN, Y.- DAI, L.- TIAN, H. et al. (2019); Treatment Of Magnesium-L-Threonate Elevates The Magnesium Level In The Cerebrospinal Fluid And Attenuates Motor Deficits And Dopamine Neuron Loss In A Mouse Model Of Parkinson's disease. *Neuropsychiatr Dis Treat.*, 15, pp. 3143-3153, https://pubmed.ncbi.nlm.nih.gov/31806980/

SLUTSKY, I. - SADEGHPOUR, S. - LI, B.- LIU, G. (2004); Enhancement of Synaptic Plasticity through Chronically Reduced Ca2+ Flux during Uncorrelated Activity, *Neuron*, 44 (5), pp. 835-849, https://pubmed.ncbi.nlm.nih.gov/15572114/

SLUTSKY, I – ABUMARIA, N.- WU, L.J.- HUANG, C.- ZHANG, L.-LI, B.- ZHAO, X.- GOVINDARAJAN, A.- ZHAO, M.G.- ZHUO, M.-TONEGAWA, S.- LIU, G. (2010); Enhancement of learning and memory by elevating brain magnesium. Neuron, 65 (2), pp. 165- 177, https://pubmed.ncbi.nlm.nih.gov/20152124/

STROEBEL, D. - CASADO, M. - PAOLETTI, P. (2018); Triheteromeric NMDA receptors: From structure to synaptic physiology. *Curr. Opin. Physiol.*, 2, pp.1–12, https://pubmed.ncbi.nlm.nih.gov/29682629/

STOHR,J- WATTS,J.C.- MENSINGER,Z.L.- OEHLER, A.- GRILLO, S.K.- DeARMOND, S.J.- PRUSINER, S.B.- GILES, K. (2012); Purified and synthetic Alzheimer's amyloid beta (Aβ) prions. *PNAS*, 109(27), pp. 11025- 11030, https://pubmed.ncbi.nlm.nih.gov/22711819/

SUN, Q. - WEINGER, J.G. - MAO, F. - LIU, G.(2016); Regulation of structural and functional synapse density by L-threonate through modulation of intraneuronal magnesium concentration. *Neuropharmacology.* 108, pp. 426–439, https://pubmed.ncbi.nlm.nih.gov/27178134/

TOFFA, D.H.- MAGNEROU, M.A.- KASSAB, A.- HASSANE, D.- SOW, A.D. (2019); Can magnesium reduce central neurodegeneration in Alzheimer's disease? Basic evidences and research needs. *Neurochem Int*. , 126, pp. 195-202, https://pubmed.ncbi.nlm.nih.gov/30905744/

WALKER,LC.- JUCKER,M.(2015); Neurodegenerative diseases: expanding the prion concept. *Annu Rev Neurosci*., 38, pp. 87- 103, https://pubmed.ncbi.nlm.nih.gov/25840008/

WANG, R.- REDDY, P.H. (2017); W*J. Alzheimers Dis.*, 57 (4), pp. 1041- 48, https://pubmed.ncbi.nlm.nih.gov/27662322/

WONG, G.K.C. - LAM, C.W.K. - CHAN, M.T.V. - GIN, T. - POON, W.S. (2009); The effect of hypermagnesemic treatment on cerebrospinal fluid magnesium level in patients with aneurysmal subarachnoid hemorrhage. *Magnes. Res.*, 22, pp. 60–65, https://citeseerx.ist.psu.edu/viewdoc/download?doi=10.1.1.905.7777&rep=rep1&type=pdf

WYLIE, M. J.- , J. P. FONTENOT, J.P.- GREENE, L.W. (1985); Absorption of magnesium and other macrominerals in sheep infused with potassium in different parts of the digestive tract. *J. Anim. Sci.*, 61, pp. 1219–1229, https://pubmed.ncbi.nlm.nih.gov/4077768/

XIONG, W. - LIANG, Y. - Li, X. - LIU, G. - WANG, Z. (2019); Direct quantitative analysis of erythrocyte intracellular ionized magnesium in physiological and pathological conditions. *Biol. Pharm. Bull*. , 42, pp. 357–364, https://pubmed.ncbi.nlm.nih.gov/30828068/

YAMANAKA, R.- SHINDO, Y.- OKA, K. (2019); Magnesium is a key player in neuronal maturation and neuropathology. *Int J Mol Sci*., 20 (14), p. 349, https://pubmed.ncbi.nlm.nih.gov/31336935/

HLÁSNÝ, J. (2020); Is magnesium deficiency the cause of neurodegeneration in animals and humans? 3rd part; Alzheimer's disease and magnesium – parasympathetic dysfunction. Výzkum v chovu skotu, 62 (3), pp. 46- 60
https://www.vuchs.cz/main/bulletin/vyzkum-v-chovu-skotu-3-2020/

In the previous study, it was warned that therapeutic administration of magnesium (Mg) is still controversial regarding the treatment of Alzheimer´s disease (AD), when higher doses of Mg may have potential detrimental side effects. The predominance of parasympathetic dysfunction in mild cognitive impairment suggests that neurodegeneration may be due to an early cholinergic deficiency that involves central autonomic network in dementia. In AD there is a decrease in acetylcholine synthesis and impaired cortical cholinergic function in patients with Alzheimer dementia. The current therapeutic arsenal of AD comprise of two classes of medications: the cholinesterase inhibitors (ChEIs), used in mild to moderate AD, and the non-competitive NMDA receptor antagonist memantine (approved for use in moderate to severe AD). So ChEIs in dementia provide symptomatic relief by inhibiting cholinesterase at synaptic cleft and increasing cholinergic transmission. However, the mechanism of action of memantine is distinct from those of cholinergic agents and is proposed to be neuroprotective. Combining ChEIs and memantine could offer greater benefits on behavior, cognition, and global outcomes (Deardorff and Grossberg, 2016). However, these drugs provide only symptomatic benefits in AD.

Cholinergic system is damaged in the brains of people with Alzheimer's disease (AD)

Components of the central autonomic network attract the greatest neurofibrillary degeneration and related cell death during the course of AD. The insular cortex and brainstem are affected from the early stages of disease. Acetylcholine, the main neurotransmitter of the parasympathetic system may be deficient in mild cognitive impairment (MCI). Hence, autonomic dysfunction may be a novel biomarker of neurodegeneration. Autonomic dysfunction, particularly parasympathetic dysfunction (PD) is prevalent in MCI. The predominance of PD in MCI suggests that neurodegeneration may be due to an early cholinergic deficiency that involves central autonomic network in dementia (Collins et al., 2012). In AD patients, relative depression of

parasympathetic activity has been reported to be associated with cognitive dysfunction. However, increased or unchanged sympathetic functions have also been reported in AD (Toledo and Junqueira, 2010).

The cholinergic system is a crucial regulator of the cardiovascular and autonomic functions, and it is prominently affected in AD, beginning in the preclinical phases. Various lines of evidence indicate that in AD the cortical perivascular cholinergic nerve terminals are largely lost, contributing to the impairment of the observed reduction in cerebral blood flow: the analysis of this relationship has also led to the cholinergic-vascular hypothesis (Claassen and Jansen, 2006; Van Beek and Claassen, 2011). The loss of parasympathetic function unmasks the baseline symphathetic bias inherent in the end-organs, resulting in the familiar signs of aging including tachycardia, constipation, insomnia, erectile dysfunction, fluid retention, and systemic inflamantion. Thes consequences in turn may contribute to many of the common diseases associated with aging including type-2 diabetes, Alzheimer´s, atherosclerosis, and cancer. Maintenance and resoration of the parasympathetic function may enable upstream control over the deleterious aspects of inherent end-organ adrenergic bias (LEE et al., 2004).

It is long-term known that an excess of Mg ions blocks synaptic transmission through the ganglia of the sympathetic nervous system of mammals (Stanbury, 1948; Hutter and Kostial, 1954). It is also accepted that peripheral parasympathetic pathways contain relay synapses at or near the target organ. The pharmacological properties of the junction between pre and post-ganglionic neurones in the parasympathetic system are believed to be similar to those in the ganglia of the orthosympathetic systém (Somjen and Baskerville, 1968). Stanbury (1948), however, failed to observe blockade of the inhibitory effect of the vagus in cats treated with sufficient Mg to block transmission in orthosympathetic ganglia.

Dietary Mg supplementation has reduced the effect of stress by reducing plasma cortisol and catecholamine concentrations. Mg may reduce neuromuscular stimulation by antagonizing Ca and reducing the secretion of acetylcholine by motor nerve impulses (HUBBARD, 1973). Mg may also reduce the release of the catecholamines from nerve terminals and the adrenal glands (CLASSEN et al., 1983). Murasato et al. (1999) suggested that Mg deficiency induces sympathetic excitation and enhances the sensitivity of the sinus node to autonomic regulation. However, the dosage of Mg in the human diet is important, as well as in the prevention of AD, as recently found in the

evaluation of two studies. If the Mg intake was lower - only within the recommended intake, then the prevention of AD was successful (Lo et al., 2019), but if the Mg intake was too high, then the prevention of AD was unsuccessful (Kieboom et al., 2017).

NOTE; Recommended dietary allowances (RDAs) for Mg, people 31+ years; male 420 mg/day, female 320 mg/day. However, large, cross-sectional, population-based data sets confirm over half the adult population in the United States does not consume adequate amounts of Mg (Tarleton, 2018). Mg absorption decreases with age. Around the age of 70, it becomes two-thirds of what is usually is with people around 30 (Seelig, 1981).

Autonomic nervous system (ANS); function according to medical textbooks

The ANS is the part of the nervous system of the higher life forms that is not consciously controlled. It is commonly divided into two usually antagonistic subsystems: the sympathetic and parasympathetic nervous system, and involves the homeostasis of organs and physiological functions. In general, the parasympathetic nervous system (PNS) is involved with digestion and energy conservation, while the sympathetic (SNS) nervous system is involved with energy expenditure and the 'fight or flight' response.

A/ Function

The ANS regulates bodily functions and the activity of specific organs. As examples, the ANS plays a role in the diameter of blood vessels, heart rate, force of contraction of the heart, diameter of the pupils, salivation, perspiration, bronchiole diameter, peristaltic movements in the intestine, spinctor diameter, erection, ejaculation, and parturition. The SNS and PNS often have opposing effects in the same organs or physiological systems, and the ANS is a major factor in homeostasis.

The SNS is frequently referred to as the "fight or flight" system, as it has a stimulating effect on organs and physiological systems. For example, the SNS constricts blood vessels feeding blood to the GI tract and skin, while dilating skeletal muscle and lung blood vessels. Bronchioles also dilate allowing more oxygen to be exchanged at the lungs. At the same time, the SNS increases heart rate and contractility of the heart. This vastly increases blood flow to the skeletal muscles and diverts blood away from organs such as the GI tract which are not important during the "fight or flight" response. Sympathetic nerves also

dilate the pupils and relax the lenses, allowing more light to enter the eyes and enabling one to see further.

The PNS has sometimes been called the "rest and digest" response. The PNS slows and relaxes many functions of organs and body systems. For example, the PNS will dilate blood vessels to the gastrointestinal (GI) tract, while slowing the heart beat and decreasing the force of the heart's contractions. These effects help to lower the metabolic strain on the body, resulting in energy conservation. The PNS can divert blood back to the skin and the gastrointestinal tract. Increased blood flow to the GI tract aids digestion. The PNS also constricts the bronchioles when the need for oxygen has diminished. During accomodation, the PNS causes the constriction of the pupils and lenses. The PNS stimulates salivary gland secretion, and accelerates peristalsis, so although the PNS generally has a calming effect on the body, it does stimulate activity too.

B/ ANS and body organs inervation

The cell bodies of preganglionic autonomic nerve cells are situated in the central nervous system (CNS). Those of the sympathetic nervous system arise in the thoracic and lumbar segments of the spinal cord. The preganglionic parasympathetic cell bodies are situated in the brain stem (cranial parasympathetic) and in the sacral spinal cord (sacral parasympathetic).

In order to reach the target organs and glands, the axons of neurons in the SNS and PNS often must travel long distances in the body. In the SNS and PNS, neurons from the CNS synapse at ganglions; a site where a group of neurons of similar function (called presynaptic neurons) connect to another group of neurons (called postsynaptic neurons), by means of a synapse. Ganglions allow for the modulation of the presynaptic input before it is sent along the postsynaptic neurons to their effector sites.

The main neurotransmitter that is located at the ganglion is acetylcholine. Acetylcholine is released from the presynaptic neuron and acts on postsynaptic nicotinic receptors in both the SNS and PNS. Postsynaptic cells pass signals to the effector organs. At the effector organs, SNS postsynaptic neurons release noradrenaline (norepinephrine) to act on adrenoceptors, with the exception of the sweat glands and the adrenal medulla. At sweat glands, the neurotransmitter is acetylcholine, which acts on muscarinic receptors. At the adrenal cortex, there is no postsynapic neuron. Instead the presynaptic neuron releases acetylcholine to act on nicotinic receptors. Stimulation of the adrenal medulla releases adrenaline (epinephrine) into the bloodstream which will act on adrenoceptors, producing a widespread increase in sympathetic activity.

In the PNS, all postsynaptic cells use acetylcholine as a neurotransmitter, to stimulate muscarinic receptors. The sympathetic axons build a chain of 22 ganglia, the so-called paravertebral ganglia, on each side of the spinal column. From these the splanchnic nerves run to the prevertebral ganglia, which lie in front of the aorta, at the level where its unpaired visceral arteries branch off. The left and right trunks of the sympathetic nerve fuse to form an unpaired ganglion in the pelvic area. Organs innervated by sympathetic fibres include the heart, lungs, esophagus, stomach, small and large intestine, liver, gallbladder and genital organs. These organs are also innervated by the part side of the parasympathetic nervous system. The digestive system distal to the lower part of the colon is regulated by the sacral parasympathetic fibres via the pelvic ganglia. The more proximal digestive tract is controlled by the vagus nerve, the largest element of the cranial parasympathetic system. Like those of the vagus, other cranial parasympathetic fibers arise in the brain stem before exiting the skull with various cranial nerves, en route to the cranial parasympathetic ganglia and the innervation of the eye muscles and salivary glands.

Magnesium (Mg) as a natural calcium (Ca) antagonist about the autonomic regulation

Autonomic dysregulation leads to alterations in cardiac function resulting in brain hypoperfusion, seizure, and loss of consciousness. Heart rate variability (HRV), which controls cardiac function via efferent fibers to the vasculature of the heart as well as the sinoatrial node and myocardium, is an important marker for autonomic dysregulation (Lahiri et al., 2008). Some authors have suggested that both Ca and Mg levels are involved in cardiovascular diseases, including sudden cardiac death (Peacock et al., 2010; Bolland et al., 2010).

Mg is considered to be a natural Ca antagonist owing to two facts; it was approved that Mg and Ca competes with one another for the same binding site, also, Mg inhibits Ca induced programmed cell death acting as an anti-apoptotic molecule antagonizing Ca-overload-triggered apoptosis (Eilat-Adar et al., 2013). Mg^{2+} is the second most abundant intracellular cation and is also a versatile ion, which is involved in practically every major metabolic and biochemical process within the cell (de Baaij et al., 2015). Mg^{2+} is required for the production of cellular energy, cell growth and development (Li et al., 2011). Mg^{2+} is also an essential cofactor for ATP polyphosphates such as DNA and RNA and metabolic enzymes essential in nerve impulse transmission, and muscle contraction (Anghileri, 2009). In neurons, Ca^{2+} and Mg^{2+} together

plays a vital role in a variety of physiological processes, from regulating gene transcription to neuronal growth, survival and even differentiation. Interestingly, Mg^{2+} was initially identified as a powerful Ca^{2+} antagonist, despite both having similar charge and chemical properties. Moreover, Mg^{2+} also protects the neuronal cells from Ca^{2+} overload (Levitsky and Takahashi, 2013).

Previous studies have indicated a tight balance between Ca^{2+} and Mg^{2+} ions that is needed for maintaining proper physiological functions such as control of muscle movement (motor neurons).Thus, influx of both extracellular Ca^{2+} and Mg^{2+} must be tightly maintained for proper intracellular ion homeostasis as alterations in Ca^{2+} and Mg^{2+} homeostasis will alter cellular functions and possibly lead to cell death. Disturbances in Ca^{2+} homeostasis have been involved in neurodegenerative diseases such as Parkinson, Alzheimer's... ,which is mainly due to the high dependence of Ca^{2+} signaling in modulating neuronal functions. In contrast, brain Mg^{2+} levels have been shown to decline in a number of acute and chronic pathologies including neurodegeneration, traumatic brain injury, and depression (Yamamoto et al., 2007).

The compartmentalization of Mg^{2+} within the cell is a key element necessary to coordinate the regulation of pathways in which transphosphorylation reactions serves as the rate-limiting step. Additionally, Mg^{2+} also has a role in the regulation of protein synthesis, which is very sensitive to small changes of intracellular Mg^{2+} within physiological ranges and the onset of DNA synthesis is dependent on the rate of protein synthesis (Vidair and Rubin, 2005). Some studies reported that Mg^{2+} also plays a part in intracellular signaling (as does Ca^{2+}), regulation of neuronal development and modulation of electric synapses (Rubin, 2005; Rubin, 2007).

Because Ca^{2+} is normally low in most cells (less than 10^{-8} M), only a small amount of Ca^{2+} needs to muscle enter to significantly increase Ca^{2+}. This is especially true for smooth muscle cells that have small internal volumes compared with their surface area. Extracellular Ca ions in a minimum concentration of about 4 p.mil. are absolutely necessary for the release of acetylcholine (Ach) during neuromuscular transmission. If the exracellular Ca ions falls, or if the Mg ions rises, the amount of Ach released will be less and may be insufficient to cause normal neuromuscular transmission- so, neuromuscular block may occur (HUBBARD, 1973). Intracellular Mg^{2+} competes not only with Ca^{2+} but also with protons or amines ($–NH_2^+$). Protons (H^+) are typically present at concentrations of less than 10^{-7} M at pH 7 and

bind to phosphate groups with a pKa of 6.5. Mg2+ is removed from ATP when the pH decreases to 6.0, leading to significant effects on Mg2+-dependent reactions. Each intracellular organelle has a characteristic concentration of protons ([H+]), indicating that Mg2+ impacts cellular biochemical reactions in an organelle-specific manner. Indeed, intracellular [Mg2+] rhythms dynamically tune cellular biochemistry in response to the metabolic demands throughout the daily cycle (Feeney et al., 2016).

Calcium (Ca2+) acts as a ubiquitous second messenger that has achieved a well-establish role in controlling cellular functions. All cells express various Ca2+ channels, pumps, and Ca2+ binding proteins that tightly control the intracellular free Ca2+ concentration [Ca2+]i. The [Ca2+]i is maintained at low nanomolar levels, because a small increase in [Ca2+]i will result in the activation of various cellular processes, that ranging from short-term responses such as muscle contraction, secretion, and neuronal transmission to long term modulation of cell growth and gene transcription (Clapham, 2007). In addition, Devisetty et al. (2005) found that decreased serum Ca levels causes disturbances in the autonomic nervous systém.

Heart rate was affected in the connection with the exercise of horses where mares were fed with 2O mg Mg-asparate-hydrochloride per kg BW/day over a period of 8 weeks. Heart rate (HR) of the Mg group determined from ECG1 at rest (63) and ECG2 after exercise (93) were significantly lower than those of the control group (82 and l08, respectively).The observed differences between both groups might be caused by the regulating effect of Mg on the vegetative nervous system (Frischmuth, l992). Mg-deficiency in dogs has been shown to increase coronary tone and potentiate coronary vasoconstriction (Turlapaty and Altura, 1980).

Therapeutic administration of Mg is still controversial regarding the treatment of Alzheimer´s disease (AD)

Magnesium is still controversial regarding the treatment of AD, and high doses of Mg may have potential detrimental side effect (Clark and Brown, 1992; Fung et al., 1995; Hallak, 1998). Next studies suggested that, especially in the nervous system, Mg2+ plays specific roles in development, brain functions, and diseases (De Baaij et al., 2015). Because of the contradictory observations, e.g., Mg2+ is trophic or toxic, an activator or an inhibitor,

increased or decreased in the pathology of several diseases, the roles of intracellular Mg2+ and its regulatory system are controversial.

Researchers in the Netherlands have found a link between levels of Mg in the blood and the risk of developing dementia. Results from the study, from 9,569 study members with an average age of 65; both low serum Mg levels (≤0.79 mmol/L) and high serum Mg levels (≥0.90 mmol/L) were associated with an increased risk of dementia (Kieboom et al., 2017).

Among postmenopausal women (aged 65–79 years without dementia on enrolment) from the Women's Health Initiative Memory Study (WHIMS) with over 20 years of follow-up, the total Mg intake between the estimated average requirement and recommended dietary allowances was associated with a low risk of compositem mild cognitive impairment / probable dementia (MCI/PD) and mild cognitive impairment (Lo et al., 2019).

However, the predominance of parasympathetic dysfunction in mild cognitive impairment suggests that neurodegeneration may be due to an early cholinergic deficiency that involves central autonomic network in dementia (Collins et al., 2012) .

Explanation from Professor Bečka about the „Mg conflicting results"

However, there can be explanation from professor PhDr., MUDr. et MVDr. h.c. Jan Bečka (Prague 28.2.1889; Mauthausen 25.2.1942), about „Mg conflicting results". He concluded (Bečka,1935; Bečka,1936; Bečka 1936a), that the tonicity of the parasympathetic nervous system is maintained (long term „enterally") by Mg2+ and OH-: and that of the sympathetic system by Ca2+ and H+ (homeopatic doses). However, short-term „parenterally", overdosage of Ca2+ (and H+) causes the inhibition of the sympathetic and the parasympathetic nervous system action prevails. Similarly, overdosage of the Mg2+ (and OH-) causes the inhibition of the parasympathetic and the sympathetic nervous system prevails. So, he considered that the actual control is a negative feedback mechanism, and, importantly, professor Becka discovered that this mechanism is influenced by the dosage of Ca2+ and Mg2+ in connection with the acid-base state of animals.

a/ The effect of diverse magnesium salts differs according to the nature of the anions

However, according to Professor Bečka research, diverse Mg salts (enteral or parenteral), may have different effects about Ca losses by faeces, urine and influence on acid-base status; which differs according to the nature of the anions. So diverse Mg salts may samely be used for atoxic physiological nutritional supplementation, but pharmacological doses of Mg salt may induce toxicity which differs according to the nature of the anions. For example studie of the effects of $MgCl_2$ and $MgSO_4$ on the ionic transfer components through the isolated amniotic membrane has shown important differences. $MgCl_2$ interacts with all the exchangers, while the effects of $MgSO_4$ are limited to paracellular components. Mainly $MgCl_2$ increases the ionic flux ratio of this asymetric human membrane while $MgSO_4$ decreases it, with all the possible deleterious fetal consequences (Bara et al., 1994).

Among the various diseases which may induce secondary Mg deficit and which are frequently observed in elderly subjects, diabetes mellitus appears as one of the main causes of Mg depletion in aged people (Durlach et al., 1993). However, hypermagnesaemia can depress insulin levels with consequent hyperglycaemia, and neuroprotective beneficts may thereby be masked. It has been suggested that magnesium chloride has a greater propensity to cause hyperglycaemia than does Mg sulphate (Marinov et al., 1996). Similar hyperglycaemia with Mg chloride infusion was reported by Blair et al.(1989), who also failed to find evidence of neuroprotection by pretreatment Mg chloride.

b/ Some contradictory results; the efficasy of enteral Mg use in growing pigs

We reviewed these more than 60 years known findings from professor Bečka (1935), taking the porcine stress syndrome (PSS) as an example when parasympathetic tonicity is maintained by homeopatic Mg doses (Hlásný, 1999). As indicated by tachycardia, tachypnoea, hyperglycaemia, and increased blood catecholamine and cortizol concentrations the action of the sympathetic nervous system prevails in this condition.. This can be due to long term Mg-deficiency, because PSS is a hypermetabolic syndrome that produces a sustained increase of intracellular Ca^{2+} levels, and, as the PSS progresses, the combination of Mg-deficiency with acidosis, triggers circulus viciosus which continues until serum K^+ reaches cardiotoxic levels.

Improved meat quality- the effect of Mg-supplementation on the PSS

This mechanism when the parasympathetic tonicity is maintained by homeopatic Mg doses (Bečka, 1935) was confirmed by Germany researchers. Ehrenberg and Helbig (1992) investigated pigs fed 5 mg of Mg per kg b.w. throughout the fattening period (ca 0.016% of Mg supplemented in the diet) . The „long term“ prolonged administration of low-dosed Mg was found to reduce the metabolic disorders that are typical features of the PSS (pH, water-binding power and meat conductivity), and improved meat quality. Similarly, Otten et al (1993) reported that chronic low-dosed Mg supplementation during the growing and finishing period; (a) reduced plasma norepinephrine but not epinephrine (b)improved meat quality (muscle pH, conductivity values) and less pale meat (PSE) than when pigs were fed a standard diet.

However, Schaefer et al. (1993) reported that meat from pigs supplemented with Mg short- term (40 g, 5 d before slaughter), also exhibited reduced muscle temperature at 45 min after slaughter and a reduced percentage drip loss. It is clear that a high dose of Mg was used. The similar results were found (D´Souza et al.1998), when pigs (mean live weight 77 kg) were supplemented with 40 g of Mg per pig/day for 5 days before transportation to the abattoir (lower plasma norepinphrine levels, lower lactic acid in the longissimus thoracis -LT, higher the muscle pH in the LT muscle, lower percentage of drip loss). Thus, according to Professor Bečka, the prevailing sympathicotony in PSS is eliminated by "long-term lower" or "short-term higher" Mg supplementation before pigs slaughter.

Inconsistent or no effect of Mg-supplementation on the PSS

The study (Hamilton et al., 2002) was carried out in „short-term“ feeding of Mg fortified diets during 2, 3 or 5 days prior to slaughter. During the study, animals were fed at a fixed level of 2.75 kg of a standard finisher diet/day; the fortified diet contained 3.2 g/d of additional Mg (0.12% of Mg supplemented in the diet) . Results from this study suggest an inconsistent effect of short-term feeding of Mg on muscle color and drip loss in pigs. There is other „short-term“ example of supplemental Mg feeding (300, 600, or 900 mg of elemental Mg /L of drinking water) for 2 days before slaughter. Pigs were not allowed access to feed (0.13% of Mg) for 15 h before slaughter but continued to have access to experimental water treatments. However, Mg did not improve pork quality (Frederick et al., 2006).

An important challenge to improve the quality of pork is to clarify the most appropriate dose, duration and source of Mg. Addition of a high level of Mg to the diet for a few days before slaughter markedly reduces the incidence of PSS (Pettigrew and Esnaola, 2001). However, it is well known from the scientific literature that PSS results from a mutation in a Ca/channel gene that is inherited as an autosomal dominant trait, usually in Landrace pigs. It seems that this genetic cause can be enhanced by Mg-deficiency. In the feed ration of growing pigs is very large Ca : Mg ratio (NRC 1988 - 13 : 1; NRC 1998 - 12: 1), compared with the human diets- there is this ratio at the level about 3 : 1. So, the susceptibility to the Mg-deficiency is very high in the swine diets (Hlásný, 1999). Over the next 20 years, however, the supplementation of Mg in mineral mixtures for pigs has increased (personal communication), so currently there are no known publications from the world about the occurrence of PSS.

On these already forgotten relationships between Mg and Ca discovered by Professor Becka in the 1930s, were brought to the attention at 31st International Congress on the History of Veterinary Medicine (Hlásný, 2000).

Some contradictory results; the efficasy of Mg use in the clinical practice

a/ Magnesium as a calcium competitor in cardiovascular diseases

Heart disease is the main cause of death today. Half of these deaths are associated with cardiac arrhythmias and sudden deaths. Mg can produce rapid vasodilatation, hypotension and the opposite effects occur with low Mg levels. Magnesium is a Ca competitor in heart ventricular muscle due to inhibition of Ca movement into the cardial cell. It may be possible to reduce the doses of Ca antagonists by supplementing them with Mg ions. In the pilot experiments was observed that, after a 10-day period of Mg-supplementation, patients who used Ca - antagonists to control hypertension showed a decrease in systolic blood pressure of about 20 mm Hg (Nastou et al., 1994). The data obtained are consistent with the hypothesis that Mg ions play important regulatory roles in cardiovascular cellular dynamics. Mg ions appears to be pivotal in regulation of cardiac haemodynamics, vascular tone, vascular activity, lipid metabolism and prevention of free radical formation. Mg ions exerts important actions on control of Ca ions : uptake, subcellular content and distribution in smooth muscle, endothelial cells and cardiac muscle cells. The ionizable Ca and

ionizable Mg ratio appears to be an important guide for signs of peripheral vasoconstriction, ischaemia or spasm and possibly atherogenesis. (Altura and Altura, 1995).

When given at physiological doses, therapy with Mg corrects the alterations in cellular function resulting from Mg deficiency, whereas at higher dosages, which induce hypermagnesaemic levels, Mg possesses pharmacological effects, such as the inhibition of the Ca influx: this may alter the electrophysiological properties of heart cells, decrease catecholamine secretion, influence the synthesis of prostacyclin and/or alter platelet function. A ubiquitous Ca-channel blockade mechanism is the main and well-established way of action whereby Mg acts at pharmacological levels; other mechanisms may be involved as well but at present remain questionable or unsettled. On the basis of the present knowledge, beneficial effects may thus be expected from high dose intravenous Mg therapy in the setting of acute myocardial infarction with respect to mortality rates, even when there is concurrent thrombolytic therapy (Weiss and Lasserre, 1994).

Several studies have shown that the inhibitory effect of Ca entry blockers decreases with age in isolated blood vessels (Van Overloop et al., 1993: Wanstall et al., 1989: Wanstall and O´Donell, 1988). Early studies have, however, reported that high Mg potentiates the inhibitory effect of Ca antagonists on Ca - induced contraction of blood vessels (Turlapaty et al., 1981). Since aortae from aged rats were senzitive to the inhibitory effect of Mg on Ca influx, it could be suggested that Mg exerts beneficial effects, as Ca antagonist , on vascular tone in aged blood vessels. High Mg decreased the senzitivity to CaCl2 in isolated aortae from aged rats, but not from adult rats, suggesting the presence of an inhibitory effect of Mg on Ca influx in aortic smooth muscle cells from aged rats. It is established that Mg modulates vascular tone by interfering with Ca utilization in the vascular sooth muscle cell. Mg affects Ca influx and Ca release from internal stores suggesting that Mg is able to regulate the activity of vascular smooth muscle cells by competing with Ca (Altura et al., 1993: Altura et al., 1987).

ZHANG et al (l992) determined the effects of lowering Mg ions stepwise on the distribution of Ca ions in single vascular smooth muscle cells (VSMCs) cultured from canine cerebral arteries. Their results suggest that: l/Mg ions regulates intracellular free Ca ions in cerebral VSMCs, probably by modulating Ca ions entry across plasma membranes and/or release from intracellular stores. 2/ the level of ionized Mg ions probably plays an important role in regulation of cerebral vascular tone, cerebral blood flow and its distribution.

b/ Magnesium and cardiovascular function

Experimantal work in animals has shown that Mg is an important regulator of vascular tone. However, the relation between blood pressure and Mg in experimantal research is complex and not fully understood. In some but not all studies hypertension has developed in Mg-deficient rats, and in other studies Mg has prevented the blood pressure rise in spontaneously hypertensive rats. The evidence from epidemiological research of a role for Mg in the development of human hypertension is inconsistant. A fall in blood pressure in relation to i.v. Mg infusion is regularly observed and i.v. Mg is clinically used in eclampsia. Peroral Mg added in nutritional doses to patients with untreated hypertension in several small studies has not affected blood pressure significantly. However, in larger study a significant fall in blood pressure of a few mm Hg was observed. If the patients are Mg-depleted after long term thiazide therapy or treated with beta blockers a more substantial fall in blood pressure may occur. Peroral Mg in pharmacological doses has repeatedly been observed to lower blood pressure. A dose-dependent blood pressure lowering effect of peroral Mg in a dose up to 40 mmol without side effects has been described. The pathophysiological background may involve several mechanisms such as interference with catecholamines, prostacyclins or with other ions such as sodium, potassium and calcium. Recent studies indicate abnormalities in cellular ion handling resulting in high free intracellular Ca and low free intracellular Mg as a common mechanism contributing to the pathophysiology behind so-called metabolic syndrome (WESTER, 1995).

The purpose of Laurant et al. (1995) study was to determine the effect of dietary Mg supplementation on blood pressure and cardiovascular function of normotensive (NT) and mineralcorticoid -salt hypertensive (HT) rats. The rats were pair-fed for 5 weeks on a purified diet containing either normal (0.15% Mg) or Mg -supplemented diet (1.0% Mg). Magnesium supplementation significantly lowered blood pressure levels in hypertensive rats, but not in NT rats. Heart rate was not affected in either case. In vitro, the addition of physiological concentrations of Mg significantly reduced coronary spasm induced by prostaglandin F alpha 2 in human coronary arteries (Kimura et al. 1989).

Within the 1982- 1985 years , seven reports (McCARRON, ACKLEY, HARLAN, NICHMAN, GARCIA-PALMIERI, GRUCHOW, REED- „all et al.“) have identified an association between a lower consumption of dietary Ca and a higher risk of hypertension in the United States. These studies have included data from a variety of sources representing local, regional, and

national surveys that include a representative sample by age, sex, race, geographic and ethnic considerations. However accumulated evidence suggests that both an excess of Ca as well as a Ca deficiency may contribute to hypertension (Resnick et al., 1986).

It is well known that the Mg is correlated with cardiovascular function and the hypertension could be associated with low serum Mg levels (Altura and Altura, 1985). Other reports contain contradictory results, suggesting that Mg deficiency could exert a normotensive or hypertensive effect (Durlach, 1988). When levels of Ca in body tissues are analysed: increased, decreased or unchanged concentrations are found in hypertensive compared with normotensive subjects (Kesteloot and Geboers, 1982: McCarron et al., 1982). In vitro, varying amounts of Ca may potentiate both contraction and relaxation of smooth muscle (Overbeck, 1984).

c/ Magnesium and platelets aggregation, antithrombotic effects

Magnesium affects both intracellular and extracellular calcium levels by various mechanisms. This may be the main reason why parenterally administered Mg, like established calcium channel blockers, has antiarrhythmic and antithrombotic effects (Serebruany et al., 1996).

Despite a wealth of clinical experience with the use of parenteral Mg salts in the treatment of acute coronary events, the mechanism of action remains unknown (Thel and O´Connor, 1995: Durlach and Rayssiguier, 1993). If parenteral Mg is indeed a vital element in protection against myocardial reperfusion injury and thrombosis, then it should target platelets, the coagulation cascade, and favorably affect hemostasis in general. In animals, high levels of extracellular Mg in vitro, as well as intravenous supplementation ex vivo, are associated with a progressive dose dependent inhibition of platelet aggregability (Herman et al., 1970: Herzog et al., 1993). However, data on the effects of Mg deficiency on platelets are contradictory in animals. Platelets from Mg deficient and control calves and rats show no differences in adenosine diphosphate breakdown- induced platelet aggregability (Stevenson and Yoder, 1970), which was significantly increased in Mg-deficient swine. There is agreement that parenteral Mg has an inhibitory effect on platelet aggregation in swine (Serebruany et al., 1996), hamsters (Risrhi et al., 1990) , dogs (Chang et al., 1985), and rabbits (Renaud et al., 1983).

The data on the association between Mg and platelets in humans are confusing. While in vitro Mg decreases platelet aggregation (Ravn et al., 1996) and reduces platelet degranulation and surface antigen expression, other reports have indicated that Mg is essential for platelet agglutination (Satoh et al., 1993)

thrombin and collagen activation (Matsuno et al., 1993) and even could substitute for Ca in supporting aggregation. This controversy arises because of apparent conflicting results from clinical trials. The LIMIT-2 trial (2 316 patients) showed a significant mortality benefit (Woods et al., 1992;1994), whereas the ISIS-4 trial (54 824 patients) found no benefit (ISIS-4, 1995). Other observers point to methodological differences between these trials that may explain the discordant results, thus leading to the need to reexamine the claim that Mg therapy is a useful adjunct in the treatment of acute myocardial infarction (Anteman, 1995: LeLorier et al., 1997: Borzak and Ridker, 1995).

d/ Magnesium and catecholamine- acetylcholine secretion

The results with patients suffering coronary artery disease and underwent bicycle stress test (Smetana, 1995) clearly suggest that Mg-therapy suppress catecholamine secretion. The other parameters did not reveal remarkable differences comparing the data of group receiving 600 mg/ day Mg over a period of 3 weeks and the placebo group. Laurant and Berthelot (1998) concluded that their results support the recommendation that Mg administration could be increased in the elderly to prevent cardiovascular diseases . They found that high and low Mg concentrations did not influence norepinephrine-induced concentrations of isolated aortae from either adult of aged rats. However, the effects of varying Mg concentrations on in vitro catecholamine-induced contraction are contraversial. In early studies have shown that reactivity to norepinephrine decreases when Mg concentration is above 12 mM (Fujiwara et al., 1978), whereas it decreases or does not change when Mg is reduced or removed (Howell and Carrier, 1986; Rahmani et al., 1990). Laurant and Berthelot (1998) emphasise that the reasons for the discrepancy between age of adult rats are unclear but may be related to an altered vascular Ca homeostasis in aged blood vessels (Maloney and Wheeler-Clark, 1996).

Several studies have investigated the role of Mg on acetylcholine- induced relaxation in isolated blood vessels with some conflicting results. Early studies have shown that Mg is required for acetylcholine - induced relaxation of canine coronary arteries since relaxation is markedly inhibited in absence of extracellular Mg (Altura and Altura, 1987: Ku and Ann, 1991). Other studies reported lowering Mg does not modify acetylcholine- induced relaxation (Farago et al., 1991). In contrast with Laurant and Berthelot (1998) other findings, some others report that increasing Mg concentrations attenuates acetylcholine - induced relaxation in feline cerebral arteries (Farago et al., 1991). It has been shown that acetylcholine - induced relaxation is improved in

aortae from mineralocorticoid- salt hypertensive rats fed a Mg- supplemented diet (Laurant et al., 1995).

Conclusions

To date, the following Alzheimer's important prevention strategies are known; control vascular risk factors, including high blood pressure, high cholesterol and diabetes. Eat a balanced diet such as the Mediterranean diet that's rich in vegetables, fruits and lean protein. However, all these more important preventive measures are related to a sufficient intake of Mg in the human diet, which is often underestimated, especially in the elderly.

Magnesium is considered to be a natural calcium antagonist. The new wisdom now emerging is that Mg is actually the key to the body's proper assimilation and use of Ca, as well as other important nutrients. The body tends to hold calcium and either store it or recycle it again and again. Mg however, is either used up or excreted and must be replenished on a daily basis. So, even though the daily need for Ca is greater, there is much more likely to be deficient in Mg.

In previous two articles, it was pointed out that the cause of neurodegeneration in cattle (BSE) should be chronic long-term Mg deficiency and that it should be similar in humans . Unfortunately, this fact or a hypothesis has not yet been pointed out in the world scientific-professional literature. Mg is still controversial regarding the treatment of Alzheimer´s disease (AD), when higher doses of Mg may have potential detrimental side effects. The predominance of parasympathetic dysfunction in mild cognitive impairment suggests that neurodegeneration may be due to an early cholinergic deficiency that involves central autonomic network in dementia. Acetylcholine, the main neurotransmitter of the parasympathetic system may be deficient in mild cognitive impairment (MCI). Hence, autonomic dysfunction may be a novel biomarker of neurodegeneration. Autonomic dysfunction, particularly parasympathetic dysfunction (PD) is prevalent in MCI.

It has been shown that acetylcholine - induced relaxation is improved in aortae from mineralocorticoid- salt hypertensive rats fed a Mg- supplemented diet. However, several studies have investigated the role of Mg on acetylcholine- induced relaxation in isolated blood vessels with some conflicting results. Similarly about the catecholamine, the effects of varying Mg concentrations on in vitro catecholamine- induced contraction are contraversial.

Thus there are some contradictory results about the efficasy of Mg use in the clinical practice. When given at physiological doses, therapy with Mg corrects the alterations in cellular function resulting from Mg deficiency, whereas at higher dosages, which induce hypermagnesaemic levels, Mg possesses pharmacological effects, such as the inhibition of the Ca influx. However, intracellular Mg2+ competes not only with Ca2+ but also with H+ protons.

According to the research of Czech Professor Bečka, the tonicity of the parasympathetic nervous system is maintained (long term „enterally“) by Mg2+ and OH-: and that of the sympathetic system by Ca2+ and H+ (homeopatic doses). However, short-term „parenterally“, overdosage of Ca2+ (and H+) causes the inhibition of the sympathetic and the parasympathetic nervous system action prevails. Similarly, overdosage of the Mg2+ (and OH-) causes the inhibition of the parasympathetic and the sympathetic nervous system prevails. And this is exactly the situation when with higher magnesium intake, AD prevention is unsuccessful. In general, only pharmacologically lower doses of Mg (homeopathic doses) can be beneficial in preventing AD. Similarly about other diseases, when Mg intake is known to be important in these diseases prevention.

References

ALTURA,B.M.- ALTURA,B.T. (1986): Magnesium- calcium interrelationships in vascular smooth muscle. Magnes. Bull., 8, pp. 338- 350

ALTURA,B.M.- ALTURA,B.T.- CARELLA,A. et al. (1987): Mg2+- Ca2+ interaction in contractility of vascular smooth muscle: Mg2+ versus organic calcium channel blockers on myogenic tone and agonist- induced responsivenes of blood vessels. Can. J. Physiol. Pharmacol., 65, pp. 729- 745.

ALTURA,B.M.- ALTURA,B.T. (1985); New Perspectives on the Role of Magnesium in the Pathophysiology of the Cardiovascular System. II. Experimental Aspects. Magnesium, 4 (5-6), pp. 245- 271.

ALTURA,B.M.- ZHANG,A.- ALTURA,B.T. (1993): Magnesium hypertensive vascular diseases, atherogenesis, subcellular compartmentation of Ca2+ and Mg2+ and vascular contractility. Miner.Electrolyte Metab., 19, pp. 323- 336.

ALTURA,B.M.- ALTURA,B.T. (1995): Magnesium and cardiovascular biology: an important link between cardiovascular risk factors and atherogenesis. Cell.Mol.Biol.Res., 41, pp. 346- 359.

ANTEMAN,E.M. (1995): Magnesium in acute myocardial infarction. Timing is critical.(Editorial). Circulation, 92, pp. 2367- 2372.
BARA,M.-GUIET-BARA,A- DURLACH,J. (1994): Comparative effects of MgCl2 and MgSO4 on the ionic transfer components through the isolated human amniotic membrane. Magn. Res., 7, pp. 11- 16.
BEČKA,J.(1935): Therapeutické využití některých elektrolytů. Bratislavské lékařské listy, 15, sešit č.3, pp.165- 169.
BEČKA,J. (1936): Einfluss der Magnesium-Verbindungen auf die Calcium-Exkretion durch die Niere und durch den Darm. Sbor. Čsl. akad. zeměděl., pp. 368-378.
BEČKA,J. (1936a): Faktoren, die den Mineralmetabolismus zerstoren. Sbor. Čsl. akad.zeměděl., pp. 584-589.
BLAIR,J.L.- WARNER,D.S.- TODD,M.M. (1989): Effects of elevated plasma magnesium versus calcium on cerebral ischaemic injury in rats. Stroke, 20, pp. 507- 512.
BOLLAND, M.J.- AVENELL,A.- BARON. J.A. et al. (2010); Effect of calcium supplements on risk of myocardial infarction and cardiovascular events: meta analysis. BMJ, 341, c3691.
BORZAK,S.- RIDKER,P.M.(1995): Discordance between meta- analyses and large- scale randomised controlled trials. Examples from the management of AMI. Ann.Intern.Med., 123, pp. 873- 877.
CHANG,C.- VARGHESE,P.J.- DOWNEY,J.- BLOOM,S. (1985): Magnesium deficiency and myocardial size in the dog. J.Am.Coll.Cardiol., 5, pp. 280- 289.
CHOLST,I.N.- STEINBERG,S.F.- TROOPER,P.J. et al. (1984): The influence of hypermagnesemia on serum calcium and parathyroid hormone levels in human subjects. N.Engl.J.Med., 310, pp. 1221- 1225.
CLASSEN,H.G.- FISCHER,G.- MOSCHLIN,M.- TILCH,C. (1983): Cubic function between increasing dietary magnesium levels and magnesium concentration of serum and bone in young rats. Magnesium, 2, pp. 267- 271.
CLAASSEN, J.A.- JANSEN, R.W. (2006); Cholinergically mediated augmentation of cerebral perfusion in Alzheimer’s disease and related cognitive disorders: The cholinergic-vascular hypothesis. J Gerontol A Biol Sci Med Sci., 61, pp. 267-271.
COLLINS,O. -DILLON,S.- FINUCANE,C.- LAWLOR,B.- KENNY,R.A. (2012); Parasympa-thetic autonomic dysfunction is common in mild cognitive impair-ment. Neurobiol Aging, 33, pp.2324-2333.

DEARDORFF,W. J. - GROSSBERG, G. T. (2016); Pharmacotherapeutic strategies in the treatment of severe Alzheimer's disease. Expert Opin. Pharmacother.,17, pp.1789–1800.
DEVISETTY, A.- KAMBAR,C.- HANUMANTH,N, (2005); Study of effect of serum calcium levels on autonomic nervous system in pre-menstrual syndrome. IOSR-JDMS, 14(3), pp. 50-55.
D´SOUZA,D.N.- WARNER,R.D.- LEURY,B.J.- DUNSCHEA,F.R. (1998): The effect of dietary magnesium aspartate supplementation on pork quality.J.Anim.Sci., 76, pp.104-109.
DURLACH,J.- RAYSSIGUIER,Y. (1993): Fatty acid profile, fibre content and high Mg density of nuts may protect against risk of coronary heart disease events. Magn.Res., 6, pp.191-192.
DURLACH,J.- DURLACH,V.- BAC,P. et al. (1993): Mg and aging. II. Clinical data : aetiological mechanism and physiopathological consequences of magnesium deficit in the elderly. Magn.Res., 6, pp. 379-394.
Durlach, J. (1988): Magnesium in clinical practice, Publ. John Libbey London-Paris, p. 386.
EHRENBERG,A.- HELBIG,J. (1992): Influence of magnesium aspartate hydrochloride (MAH) on transportation and slaughtering stress in pigs. In: Abstracts of 4th European Congress on Magnesium (Giessen), p. 39.
EILAT-ADAR, S.-TALI,S.- CHAIM, Y.- YAAKOV,H. (2013); Nutritional Recommendations for Cardiovascular Disease Prevention. Nutrients, 5 (9), pp.3646- 83.
FEENEY, K.A.- HANSEN,L.- PUTKER,M. et al. (2016); Daily Magnesium Fluxes Regulate Cellular Timekeeping and Energy Balance. Nature, 532, pp. 375- 379.
FREDERICK,B.R.- vanHEUGTEN,E.- HANSON,D.J.- SEE,M.T. (2006): Effects of supplemental magnesium concentration of drinking water on pork quality. J.Anim.Sci., 84, pp.185-190.
FRISCHMUTH, E. (l992);Effects of a Magnesium Supplementation on heart rate and on the EEG of cross-bred horses passing the mare performance test at the Hessian Landgestüt at Dillenburg. 4th European Congress on Magnesium, Giessen.
FUJIWARA,M.- KITAGAWA,H.- KURAHAASHI,K. (1978): Effect of magnesium on contractile responses induced by electrical transmular stimulation and noradrenaline in rabbit thoracis aorta. Br.J.Pharmacol., 63, pp. 51- 55.
HAMILTON,D.N.- ELLIS,M.- HEMANN,M.D.- McKEITH,F.K.- MILLER,K.D.- PURSER,K.W. (2002): The impact of longissimus glycolytic

potential and short-term feeding of magnesium sulfate heptahydrate prior to slaughter on carcass characteristics and pork quality. J.Anim.Sci., 80(6), pp. 1586-1592.
HERZOG,W.R.- ATAR,D.- GURBEL,P.A.- VOGEL,R.A. et al. (1993): Effect of magnesium sulphate infusion on ex vivo platelet aggregation in swine. Magn.Res., 6, pp. 349-353.
HERRMAN,R.G.- LACEFIELD,W.B.- CRWE,V.G. (1970): Effect of ionic calcium and magnesiumon human platelet aggregation. Proc.Soc.Exp.Biol.Med., 135, p. 100.
HLÁSNÝ,J. (1999): Nové poznatky o předcházení stresu u prasat. Výzkum v chovu skotu (Rapotín), 41(1), pp. 22- 30.
HLÁSNÝ,J. (2000): Professor Jan Bečka and his still useful magnesium research of the thirties. In: Book of abstracts of 31st International Congress on the History of Veterinary Medicine (Brno), p. 44.
HOWELL,R.E.- CARRIER,G.O. (1986): Influence of magnesium on norepinephrine - and histamine - induced concentrations of pulmonary vascular smooth muscle. Pharmacologgy, 33, pp. 27-33.
HUBBARD,J.I. (1973): Microphysiology of vertebrate neuromuscular transmission. Physiol.Rev., 53, pp. 674- 679.
HUTTER,O.F.- KOSTIAL,K. (1954); Effect of magnesium and calcium ions on the release of acetylcholine.J Physiol., 124 (2), pp. 234-241.
ISIS- 4 (1995); Collaborative Group: ISIS- 4, a randomized factotial trial assessing early oral captopril, oral mononitrate, and intravenous magnesium sulphate in 58050 patients with suspected acute myocardial infarction. Lancet, 345, pp. 669- 685.
KESTELOOT, H.- GEBOERS,J. (1982); Calcium and Blood Pressure. Lancet, 1 (8276), pp. 813- 815.
KIMURA,T.- YASUE,H.- SAKAINO,N. et al. (1989): Effects of Mg on the tone of isolated human coronary arteries: comparison with diltiazem and nitroglycerin. Circulation, 79, pp.1118- 1124.
LAHIRI,M.K.- KANNANKERIL, P.J. - GOLDBERGER, J.J. (2008); Assessment of autonomic function in cardiovascular disease: physiological basisand prognostic implications. J Am Coll Cardiol., 51(18), pp. 1725-33.
LAURANT,P.- KANTELIP,J.P.- BERTHELOT,A. (1995): Dietary magnesium supplementation modifies blood pressure and cardiovascular function in mineralocorticoid - salt hypertensive rats but not in normotensive rats. J.Nutr., 18, pp. 830- 841.
LAURANT,P.- BERTHELOT, A. (1998): Effect of age on in vitro magnesium modulation of vascular reactivity in the rat aorta. Magn.Res., 11, pp. 71- 83.

LeLORIER,J.- GREGOIRE,G.- BENHADDAN, A. et al. (1997): Discrepancies between metaanalyses and subsequent large randomized controlled trials. N.Engl.J.Med., 337, pp. 536- 542.
LEE,Y,P.- YUN,A.J.- BAZARR,K.A. (2004): Conditions of aging as manifestations of sympathetic bias unmasked by loss of parasympathetic function. Med Hypotheses, 62, pp. 868-870.
LUTFI, M.F. (1989); Correlations between electrolytes levels and heart rate variability in apparently healthy subjects. Am J Physiol., 256(6), pp. 1573-9.
MALONEY,J.A.- WHEELER-CLARK,E.S.(1996): Reduction in sarcoplasmic reticulum Ca2+ - ATP-ase activity contributes to age - related changes in the calcium content and relaxation rat of rabbit aortic smooth muscle. J.Hypertens., 14, pp. 65- 74.
MARINOV,M.B.- HARBAUGH,K.S.- HOOPES,P.J. et al. (1996): Neuroprotective effects of preischemia intraarterial magnesium sulphate in reversible focal cerebral ischemia. J.Neurosurg., 85, pp. 117- 124.
MATSUNO, K.- KOYAMA, M.- TAKEDA,H. et al. (1993): Cytosolic free magnesium concentration in human platelets. Thromb.Res., 69, pp. 131- 137.
McCARRON,D.A.- MORRIS, C.D.- COLE,C. (1982); Dietary Calcium in Human Hypertension. Science, 217 (4556), pp.267- 269.
MURASATO,Y. - HARADA,Y.- IKEDA,M. et al. (1999); Effect of magnesium deficiency on autonomic circulatory regulation in conscious rats. Hypertension, 34(2), pp. 247–52.
NASTOU,H. - SARROS,G.- NASTOS,A.- SARROU,V.- RONTOYIANNIS, G.- THEOPHANIDES,T.- ANASTASSOPOU, J. (1994): The role of magnesium in the cardiovascular system. Magn.Res., 7, pp. 10-11.
OTTEN,W.- BERRER,A.- HARTMANN,S. et al. (1993): Effects of magnesium fumarate supplementation on catecholamines, cortizol and blood metabolites in swine. Proc. 39th Int. Congr. Meat Sci. Technol., Calgary, Canada. pp. 82-87.
OVERBECK, H.W. (1984); Attenuated Arteriolar Dilator Responses to Calcium in Genetically Hypertensive Rats. Hypertesion, 6 (5), pp. 647- 653.
PEACOCK,J.M.- OHIRA, T.- POST, W. et al. (2010); Serum magnesium and risk of sudden cardiac death in the Atherosclerosis Risk in Communities (ARIC) Study. Am Heart J , 160(3), pp. 464–70.
PETTIGREW, J.E.- ESNAOLA, A. (2001); Swine nutrition and pork quality: A review. J.Anim.Sci., 79, pp. E316- E 342.
RAHMANI,M.A.- NEVES,M.- MANGROO,T.- BENNETT,T. (1990): In vitro caffeine induced aortic smooth muscle reactivity in rat. Artery , 17, pp. 127-143.

RAVN,H.B.- VISSINGER,H.- KRISTENSEN, S.D.- HUSTED,S.E. (1996): Magnesium inhibits platelet activity- an in vitro study. Thromb.Haemost., 76, pp. 88- 93.
RENAUD,S.- CIAVATTI,M.- THEVENON,C. et al. (1983): Protective effects of dietary calcium and magnesium on platelet function and atherosclerosis in rabbits fed saturated fat. Atherosclerosis, 47, pp. 187- 198.
RESNICK,L.M.- GUPTA, R.K.- SOSA, R.E. et al. (1986); Effects of Altered Dietary Calcium Intake in Experimental Hypertension: Role of Intracellular Free Magnesium. J Hypertens Suppl., 4 (5), pp. 182- 185.
RISRHI,M.- AHMAD,A.- MAKHEJA,A. et al.(1990): Effects of reduced dietary magnesium on plateled production and function in hamsters. Lab.Invest., 63, pp. 717-721.
SATOH,S.- TAKAHASHI,K.- SAKURAGAWA,N. (1993): Thrombospodin as an agglutinin of platelets. Thromb. Res., 72, pp. 509- 517.
SCHAEFER,A.L.- MURRAY, A.C.- TONG,A.K.W. et al. (1993): The effect of ante mortem electrolyte therapy on animal physiology and meat quality in pigs segregating at the halothane gene. Can.J.Anim. Sci., 73, pp. 231- 237.
SEELIG,M.S. (1981): Magnesium requirements in human nutrition. Magnes.Bull., 3/1a, pp. 26- 54.
SEREBRUANY,V.L.- SCHLOSSBERG,M.L.- EDENBAUM,L.R. et al. 1996): Intracoronary magnesium and diltiazem affect to a similar extent certain hemostatic factors during AMI in swine. Pharmacology, 53, pp. 224- 233.
SMETANA,R. (1995): Magnesium and coronary artery disease . Magn.Res., 8, p, 68.
SOMJEN, G.G.- BASKERVILLE, E.N. (1968); Effect of Excess Magnesium on Vagal Inhibition and Acetylcholine Sensitivity of the Mammalian Heart in situ and in vitro. Nature, 217, pp. 679- 680.
STANBURY,J.B. (1948); The blocking action of magnesium ion on sympathetic ganglia. J Pharmacol., 93, pp. 52-56.
STEVENSON,M.M.- YODER,I.I. (1970): Studies on platelet aggregation, plasma edenosine diphosphate breakdown and blood coagulation in Mg-deficient calves and rats. Thromb.Diath. Haemorrh., 23, pp. 299- 305.
TARLETON, E.K. (2018); Factors influencing magnesium consumption among adults in the United States. Nutr Rev., 76 (7), pp.526- 538.
THEL, M.C.- O´CONNOR,C.M. (1995): Magnesium in acute myocardial infaction. Coron.Artery Dis., 6, pp. 831- 837.
TOLEDO, M.A. - JUNQUEIRA, L.F. Jr. (2010); Cardiac autonomic modulation and cognitive status in Alzheimer’s disease. Clin Auton Res., 20, pp.11-17.

TURLAPATY, P.D.M.V.- WEINER,R.- ALTURA,B.M. (1981): Interactions of magnesium and verapamil on tone and contractility of vascular smooth muscle. Eur.J.Pharmacol., 74,pp. 263- 272.
TURLAPATY,P.D.M.V.- ALTURA,B.M. (1980): Magnesium deficiency produces spasm of coronary arteries: relationship to etiology of sudden death ischemic heart disease. Science, 208, pp.198-200.
Van BEEK, A.H.- CLAASSEN, J. A.- OVERLOOP, B. et al. (1993); Age, endothelium and aortic reactivity to calcium in Wistar rat. Life Sci., 53, pp. 821- 831.
WANSTAL,J.C.- O´DONNELL,S.R. (1988): Inhibition of nerepinephrine contractions by dithiazem on aorta and pulmonary artery from young and aged rats: influences of alphag- adrenoceptor reserve. J.Pharmacol.Exp. Ther., 245, pp.1016- 1020.
WANSTAL,J.C.- O´DONNELL,S.R. (1989): Influence of age on calcium entry blocking drug in rat aorta is spamogen- dependent. Eur.J.Pharmacol., 159, pp. 241- 246.
WEISS,M.-LASSERRE, B. (1994); Should Magnesium Therapy Be Considered for the Treatment of Coronary Heart Disease? I. A Critical Appraisal of Current Facts and Hypotheses. Magnes Res., 7 (2), pp. 135-144.
WESTER,P.O. (1995): Magnesium and blood pressure. Magn.Res., 8, p. 77.
WOODS,K.L.- FLETCHER,S.- ROFFE,C.- HAIDER,I. (1992): Intravenous magnesium sulphate in suspected AMI: results of the second Leicester Intravenous Magnesium Intervention Trial (LIMIT-2). Lancet, 339, pp.1553-1559.
WOODS,K.L.- FLETCHER,S. (1994): Long-term outcome after intravenous magnesium sulphate in sucpected AMI: the second Leicester Intravenous Magnesium Intervention Trial (LIMIT- 2), Lancet, 343, pp.816- 819.
YAMAMOTO,S.- WAJIMA,T,- Hara,Y, et al. (2007); Transient receptor potential channels in Alzheimer disease. Biochim Biophys Acta, 1772, pp.:958-967.
ZHANG, A.- CHENG,T.P.- ALTURA, B.M. (1992); Magnesium Regulates Intracellular Free Ionized Calcium Concentration and Cell Geometry in Vascular Smooth Muscle Cells. Biochim Biophys Acta, 1134 (1), pp. 25-29.

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Classical BSE was first diagnosed in cattle in the United Kingdom (UK) in 1986, but had probably been present in the country's cattle population since the 1970s or earlier Scientists believe that cattle are usually infected through the dietary intake of prion contaminated feed during their first year of life. The risk of contamination occurs if the feed contains products derived from ruminants, such as meat-and-bone meal (MBM).. It has then been reported in 25 countries other than the UK, mainly in Europe, Asia, the Middle East and North America (OIE, 2018).

There is the spatiotemporal correlation of BSE to a novel form of human prion disease, variant Creutzfeldt-Jakob disease (vCJD), which led to the classification of BSE as a zoonotic disease (and the "cause" of vCJD) in 1996. Most reported vCJD cases appear to have been infected through the consumption of bovine meat products contaminated with the agent of BSE. In three cases, reported by the UK, the mode of transmission is thought to be through receipt of blood from an asymptomatic, infected donor. Causative agents of vCJD are prions, composed of misfolded prion proteins (PrPSc), which form aggregates in neurological tissue leading to progressive brain damage and characteristic signs and symptoms of the disease. Since 1996 and as of August 2013, a total of 229 cases of vCJD cases have been identified from 11 countries: 177 from the UK and 52 cases elsewhere (ECDC, 2017).

Saving the rainforest

Recently, six European supermarket chains, said they would stop selling some or all beef products from Brazil due to links with destruction of the Amazon rainforest. The pledges ranged from supermarket chain Lidl Netherlands, which committed to stop selling all beef originating in South America starting in 2022. (Reuters, 2021).

In response to the European BSE crisis in the 1990s the European Commission (EC) adopted „Regulation EC No 999/2001"of 22 May 2001 and installed a ban on the use meat and bone meal (MBM) in feed for the all farmed animals . When MBM had to be taken out of the feed it was replaced by

soybean products, which needed to be imported mostly from Brazil . Because of the vast demand from Europe large areas were cultivated to satisfy the European demand. Because of the MBM ban in Europe, annually ca 18 million tonnes of MBM is replaced by ca 25 million tonnes of soybeans. Since the ban on MBM was installed the area planted with soybeans has increased from 10 million hectares in the eighties to more than 20 million hectares at the beginning of this century.

The research, published in the journal Science found that roughly 20% of soya exports and at least 17% of beef exports to the EU may be "contaminated with illegal deforestation", the researchers said. According to their analysis, two million tons of soya grown on properties with illegal deforestation may have reached EU markets annually during the period of analysis, 500,000 of which came from the Amazon (Briggs, 2020). So apart from the nutritional benefits putting MBM back into the feed this would also have an enormous environmental benefit as it would put less pressure on the rainforest in South America.

MBM as the „infectious“cause of BSE in the United Kingdom (UK) was already questioned in 2000 (The BSE Inquiry)

The cause of BSE (BSE Inquiry, 2000)

The BSE Inquiry was announced in Parliament on 22 December 1997, and set up on 12 January 1998, to establish and review the history of the emergence and identification of BSE and new variant CJD in the United Kingdom. Classical BSE was first diagnosed in cattle in the United Kingdom (UK) in 1986.

Dr. Wilesmith became the main investigator of the cause of BSE in the UK. He joined the Central Veterinary Laboratory ("CVL"), from1985 to 1986 he was a Senior Research Officer in the Epidemiology Department , and in 1986 he became head of that Department. His general role as head of the Epidemiology Department was to maintain the day-to-day running of the Department and maintain and develop expertise in veterinary epidemiology. From 1987 he have been responsible for epidemiological research on BSE and other animal TSEs in the United Kingdom (UK)

Wilesmith et al., 1988 and Wilesmith et al., 1991, were firmly of the view that BSE was an extended common source epidemic. This was because BSE appeared in most parts of Great Britain within a short space of time, shorter than the mean incubation period of BSE. By the end of 1987 John Wilesmith,

the Head of the CVL Epidemiology Department, had concluded that the cause of the reported cases of BSE was the consumption of MBM, which was made from animal carcasses and incorporated in cattle feed. This conclusion was correct. It had been reached with commendable speed. The following provisional conclusions of Mr Wilesmith, which were generally accepted at the time as a basis for action, were reasonable but fallacious:

- the cases identified between 1986 and 1988 were index (ie, first generation) cases of BSE;

- the source of infection in the MBM was tissues derived from sheep infected with conventional scrapie;
- the MBM had become infectious because rendering methods which had previously inactivated the conventional scrapie agent had been changed.

The cases of BSE identified between 1986 and 1988 were not index cases, nor were they the result of the transmission of scrapie. They were the consequences of recycling of cattle infected with BSE itself. The BSE agent was spread in MBM. BSE probably originated from a novel source early in the 1970s, possibly a cow or other animal that developed disease as a consequence of a gene mutation. The origin of the disease will probably never be known with certainty.The theory that BSE resulted from changes in rendering methods has no validity. Rendering methods have never been capable of completely inactivating TSEs.

The BSE Inquiry (2000), agreed that MBM was the major vector of BSE in cattle, including via unintentional cross-contamination of ruminant diets with feed, or MBM, intended only for monogastric species.

WHY unintentional cross-contamination of ruminant diets ?

In the early 1980s there were in the UK about 400 feed companies, although this number was in decline. Feed manufacturers produced both ready-to-use compound feeds and protein concentrates which farmers could use if they preferred to mix their own feed on the farm.

At first; Wilesmith made (July 1987) contact with Mr Clive Scott and Alan Adamson, who were nutritionists with ADAS in Bristol, to obtain information on recent changes in the ingredients used in concentrates produced for cattle. He recall that at this time three ingredients (manioch, rape seed and maize

gluten) were of interest as there was some indication that their use had increased.

At second; During January and February 1988 Mr. Wilesmith and others began to examine the use of MBM and tallow in cattle feed. For example he and Mr Keith Meldrum (Deputy CVO) met with Dr Laurson-Jones, a veterinary colleague in the feed industry in early January 1988. At this time he received from Mr Gallehawk the data which he had requested on the composition of livestock rations. This showed that between 1979 and 1987, there had been no increase or new use of MBM in cattle feed and led he to consider whether any of the processes in the animal feed industry had changed since MBM had been included in animal feed (Wilesmith, 2000).

So between 1979 and 1987, there had been no increase or new use of MBM in cattle feed in the UK. But then it is not possible for perhaps millions of British cows to be fed MBM, it would have to be a huge increase in MBM consumption! However, „maize gluten“ (not MBM) as a source of protein was fed, which is still a common practice today. See an example from that time, published in the scientific British veterinary journal; Fourteen cases of bovine spongiform encephalopathy (BSE) were diagnosed on the basis of clinical examination in a closed herd of British Friesian cows (ca 500 cows) during a 9-month period from October 1987 until June 1988. No protein of animal origin had been fed to either heifers or cows in this herd during the past 5 years, only higher protein „maize gluten“ was fed in higher doses (Winter et al., 1989).

Wilesmith carried out calculations which indicated that the exposure of the cattle population to the BSE agent was likely to have begun in the winter of 1981-82. Had anything occurred at about this time to explain the disease? Further investigations were put in hand to explore, with the help of the feed and rendering industries, why it might be that cattle feed had suddenly started infecting cattle (BSE Inquiry, 2000).

NOTE; So the extensive BSE Inquiry (text of more than 4,000 pages) points out that the origin of BSE from sheep or the spread of BSE after changes in rendering practices no longer applies and does not even mention direct-targeted feeding of MBM to cattle (cows). When it comes to unintentional cross-contamination of ruminant diets with feed, or MBM, intended only for monogastric species.

Indeed, did something important happen at that time (1981-82) regarding cattle nutrition that would explain the disease?

In Britain the average lactating cow receives about 50% of her nutrients from concentrates. Protein concentrates are propriety-products specially designated for futher mixing before feeding at an inclusion rate to 5% or more, with planned proportions of cereals and other feedingstuffs. Protein concentrates contain blended high protein ingredients such as fish meal and soyabean meal fortified with essential minerals, vitamins… Prior to 1979 (ARC, 1965) the best estimates for protein in ruminants were expressed as digestible crude protein (DCP). However, the DCP systém does not express the protein requirements for high- yielding cows at all adequately. The New Protein System by the ARC (1980) is based on the use rumen degradable protein (RDP) and rumen undegradable dietary protein (UDP). It follous that, in a case of dairy cattle fed a high protein materials of low degradability , such as fish meal or blood meal there is likely to be „room“ in the total ration for added non protein nitrogen (NPN), and some of this might be provided as urea… However, it is likely that for some years both the new protein systém, utilizing the concepts of RDP and UDP, and the old DCP systém will run side- by- side (Wilson and Brigstocket, 1981).

However, this new feeding systém (ARC, 1980), as an official proposal for the UK, (see the publication; 351 pages) grossly overstimated the requirement of cattle for the protein (Alderman and Cottrill, 1993). And apparently indeed, as predicted by the aforementioned scientists, both systems were used. However, according to the first system (1965), the feeding-administration of the required UDP (1980) was not respected, with a higher protein intake. Higher protein intake - high level of ammonia in the rumen - without adequate supply of magnesium to the cattle feed ration, this results to hypamagnesaemia. The lack of magnesium (Mg) with the simultaneous protein excess in the diet concentrates to the Mg- deficiency in the ruminant tissues.

The Mg- deficiency among British cows has been known since the 1960s. In early 1980s , it was stated that in most circumstances there is no safe alternative to providing extra dietary Mg, with daily 30 g of available Mg per lactating cow being an average target. Because mostly were available only commercial Mg-blocks with very considerable variation in palatability, mostly very low Mg intake (Whitaker et al., 1985). There was a substantial increase (England and Wales) in the numbers of cases of clinical hypo-Mg in dairy cows in spring 1984 when 1 per cent were affected in May 1984 and 0.9 per cent in June (250 herds, average size 130 cows). This was two and half times greater than the

May average for the previous three years and five times greater than the June average. Interpreted as a similar percentage of the United Kingdom national herd , there probably were 64.000 cases of clinical hypo-Mg in this two month period of 1984 (Whitaker et al., 1985).

Through the agency of DHHPS , which monitors 30.000 cows (1992), it has become apparent that the incidence of clinical and subclinical hypomagnesiamia in dairy cows seems to be still high. Farmers are reporting losses and a significant proportion of blood samples are showing low Mg levels. Since the early 1990s, it was Mg gradually implemented in concentrates. As stated by Whitaker et al. (1993) the most realiable method of Mg- deficiency prevention is by feeding Mg in the daily feed ration concentrate allowance.All these recommendations point to a change in the early 1990s, an increase in Mg supplementation to British cows, and a decrease of Mg deficiency. Therefore if we will put this „phenomenon“ into practice; significantly higher additional dietary Mg-supplementation - can be a cause about the BSE incidence decrease in the UK, after 1993/94 period, according to the alternative "BSE magnesium- ammonia" theory (Hlásný, 2000).

Does publication (BMJ journal, April 2001) contributed to the ban on feeding MKM in EU countries?

American scientist, Paul Brown, wrote in early April 2001 (BMJ) that the infectious agent that causes scrapie in sheep had crossed the bovine species barrier and caused BSE. He emphasizes that there is one indisputable fact in the story of BSE and vCJD and that BSE is the cause of vCJD. From an epidemiological point of view, BSE is a classic epidemic and will undoubtedly become a textbook example for students (Brown, 2001). Scientist Brown consolidated his hypotheses for students with a picture of the MKM ban on ruminants (1988) and the decline in BSE (1993). Ferguson-Smith responded to the BMJ in June 2001, saying; does Brown believe that scrapie “has mysteriously chosen the United Kingdom as its only geographical site and the early 1980s as its only historical occurrence” to appear in cattle? He explains that the comparatively high incidence of scrapie in sheep in the United Kingdom and the changes in rendering were responsible. This seems implausible, for reasons set out in the report of the BSE Inquiry (2000).

I am not aware of comparative data on the incidence of scrapie in the United Kingdom and abroad. Similar changes in rendering were occurring elsewhere, and no rendering process either before or since the emergence of bovine spongiform encephalopathy has been capable of completely inactivating scrapie. The BSE inquiry was impressed by the more plausible alternative view that the agent giving rise to bovine spongiform encephalopathy was a novel strain of unknown origin that was pathogenic to humans. This view held that the strain arose in southern England in the 1970s and that the infection spread in waves through recycled infected cattle waste in meat and bonemeal. We will probably never know the origin of the agent—whether it arose in cattle or sheep or, indeed, any other species whose waste was incorporated into meat and bonemeal (Ferguson-Smith, 2001). However, Ferguson-Smith's argument (June 2001) was not very valid because the EC apparently succumbed to the aforementioned "pressures" from Brown and already in May 2001 "decided" to ban the feeding of MBM to all european livestock (Regulation (EC) No 999/2001).

NOTE; Professor Malcolm Ferguson- Smith was appointed (1998) as the main head scientist member of Lord Phillips' Committee to review the UK Government's original BSE inquiry and consider the emergence of BSE and new variant CJD and the actions taken, reporting in BSE Inquiry (2000).

Bovine spongiform encephalopathy (BSE) and variant Creutzfeldt-Jakob disease (vCJD), a brief overview of British history up to 2007, from British journalists

When the news first broke that 'mad cow disease' could be passed to people, some scientists predicted that tens of thousands of us could eventually die of vCJD, the human form of BSE. Ten years on, the death toll stands at 160. So has the real danger passed? Or are many of us still carrying the disease unknowingly? Ian Sample talks to the scientists most closely involved in the crisis and learns that the real threat now is not from cows - but from other humans (Sample, 2007).

Initial doubts of a top well-known medical expert (BBC 2000) and British minister, as well as other experts about the origins of BSE and vCJD

The announcement of the news, on March 20 1996, fell to Tory health secretary Stephen Dorrell and caused more than a slashing of beef prices at supermarkets. Many were furious about the Conservative government's handling of the dangers of eating BSE-contaminated meat. They had heard the chief medical officer, Sir Donald Acheson, declare categorically that beef was safe for everyone to eat, a message repeated by his successor. They had seen John Gummer, then agriculture minister, feed his four-year-old daughter, Cordelia, a beefburger in front of cameras in Ipswich. Confidence in the government, and to a lesser extent the scientists it consulted, evaporated.

In the absence of hard facts, speculation over the eventual human death toll reached apocalyptic proportions. The doom-laden predictions now look to be well off the mark, but has the threat really passed? More than 10 years since Dorrell's announcement, and 20 years since the first cases of BSE were identified, at least 160 people have died from vCJD and more are expected to die soon. But despite billions spent on efforts to save Britain's beef industry and protect its citizens, all the major questions remain unanswered. The origin of the disease? A mystery. The number of people infected with vCJD? A mystery.

The risk that those harbouring the disease will infect others? Again, a mystery. And since there is still no blood test and no cure, the final death toll is anyone's guess. Right now, the only sure-fire test for the disease involves examining chunks of people's brains or other internal organs, and so is usually only performed on the dead.

The story of BSE in Britain is a case study in the ruthless efficiency of intensive farming, the self-serving behaviour of government departments and the patronising caution extended to the public when explaining risk. It reveals the impotence of the scientists involved - at least at the outset, when they were being called upon to give meaningful advice while still battling to understand a disease they had never encountered before. At the end of 1986, pathologists at the Central Veterinary Laboratory were analysing slivers of brain tissue sliced from cattle that appeared to have contracted a new disease. It left the cattle uncoordinated and jerky, and ultimately proved fatal. Under a microscope, the brain damage resembled scrapie, a disease caused by rogue proteins known as "prions" that had been endemic in the national sheep flock for nearly 200 years.

Without publicity, an investigation was launched to find the cause of the outbreak. It revealed an alarmingly widespread disease. One year later, 95 cases of BSE had been confirmed on 80 farms. By February 1988, 264 cases had been tracked back to 223 farms. The number of cases began to grow exponentially. "It was turning into a major crisis and there was all sorts of wild guesswork going on because no one understood it," says Chris Higgins, who now chairs

the government's advisory committee on spongiform encephalopathy diseases. "The politicians didn't know what to do and the scientists didn't know what to do. We didn't know where it came from, what caused it, how bad it might be. We didn't know anything."

Initial confusions about the origin of BSE has been "explained" by four British veterinarians (Wilesmith et al., 1988)

John Wilesmith, an epidemiologist and lead investigator at the Central Veterinary Laboratory, suspected that cattle feed - the common factor linking the cases - was to blame. Specifically, he proposed that scrapie-infected sheep offal had been mixed into meat-and-bone meal, a nutritious food source that is made by grinding down and baking a slurry of sheep and cow remains. The process had been perfected by the rendering industry (which uses every scrap of the animal carcass for various products) during an efficiency drive in the aftermath of the second world war.

The investigation led to the government imposing what the cattle industry regarded, at the time, as a draconian measure - a ban on the use of certain meat-and-bone meal in feed for cattle and sheep. The ban was followed by compulsory orders to slaughter all animals showing signs of BSE. The moves were made to protect cattle, but the possibility of risk to the public had not gone unnoticed.

As events gathered pace, Acheson, the chief medical officer, set up a working party, headed by Sir Richard Southwood, an Oxford University zoologist, to advise on the implications of BSE. When the working party reported back in early 1989, it agreed that scrapie-contaminated meat and bone meal was the most likely cause of the BSE outbreak. It advised that baby-food manufacturers should stop using cow and sheep offal, especially the thymus (a gland known to be highly infected by scrapie prions), in their meat-based meals. But it still maintained that it was "most unlikely BSE would have any implications for human health".

Officials at what was then the Ministry of Agriculture, Fisheries and Food (Maff) were unsettled. If potentially infected offal from cows was not considered safe for babies, why should it be safe for adults? After wrangling with the Department of Health, a blanket ban on cow offal entering the food chain was introduced, but not before health secretary Kenneth Clarke's officials recommended he let Maff take sole responsibility for the move. They feared that the ban was going beyond the scientific advice of the Southwood committee, which maintained there was little cause for alarm.

British veterinarians began laboratory examining the heads of thousands of cows... (without the interest about MBM feeding)

On farms around Britain, veterinary inspectors were stretched to breaking point. Some were visiting four farms a day, making diagnoses, completing paperwork and arranging the slaughter and destruction of animals. The carcasses mounted up. Government had made the decision to leave the building of new incinerators to the private sector and many were held up by difficulties with planning permission. The carcasses that could not be incinerated were dumped in landfills.

At the Central Veterinary Laboratory and other sites, where cattle heads were being sent for brain-tissue analysis, scientists faced a growing backlog, despite working around the clock. Cases of BSE continued to rise, and by the end of 1989, nearly 10,000 cattle at more than 5,000 farms had tested positive.

The detection of BSE in a cat, first fear of transmission of BSE to humans… however, only an isolated and "non-recurring" finding (Leggett et al., 1990)

Within government, confidence that bans on offal and MBM would stem the rise in new BSE cases began to build and efforts shifted toward lifting the restrictions on British beef which had been imposed by other countries, including the European Union. But in April 1990, a Siamese cat in Bristol was to trigger a new wave of concern. Two vets at the city's university diagnosed the cat as having a feline form of BSE. While the government maintained the best course of action was to reassert the safety of eating beef, scientists knew the discovery was a turning point.

"The argument that this disease was going to be safe to humans was based on it coming from scrapie, even though it hadn't caused problems before in humans," says John Collinge, director of the Medical Research Council's prion unit at University College, London. "When domestic cats started going down with it, we knew the disease had a new host range, and you couldn't say anything about whether humans were or weren't at risk. The problem was that people who stood up and said there might be a risk were quite severely leant on."

Late in 1990, it became clear that the ban on meat-and-bone meal was beginning to have an effect. The rate of increase of new BSE cases began to slow down. Banning the feed turned out to be the single most important

measure in stemming the tide of BSE. "Getting that ban in place so quickly, even though it was incomplete, was remarkably effective," says Danny Matthews at the Veterinary Laboratories Agency in Weybridge. "When you could see what sort of tsunami was coming up, if we hadn't got the ban in place then, we really would have been in a disastrous situation 12 months later."

But fears that the disease might still strike humans were growing. Enormous quantities of BSE-contaminated beef had started pouring into the food chain in 1986. The incubation period in cattle meant that infected but outwardly healthy cattle had been processed into steaks, burgers, pet food and baby food. And scientists knew the disease, were it able to jump into humans, would take time to appear.

Researchers have begun experimenting with feeding infected brain tissue but still without experiments with MBM feeding

Behind the scenes, researchers were frantically trying to understand the infection. The consensus was that a new type of rogue prion was to blame. When it infected other animals, it forced healthy prions to change, and then spread the disease. Many experiments were insightful but not encouraging. In one test, sheep resistant to a major strain of scrapie contracted BSE after being fed just half a gram of infected cow brain tissue. Another test proved BSE could infect primates. Despite an emergency meeting of scientists to discuss the primate study, government assurances maintained that the risk to humans was remote. What they failed to make clear was that the scientists only felt the risk was low because of the raft of bans already in place. The studies made it abundantly clear that there was a grave risk for people who had already eaten infected meat.

The scientists' worries intensified when random inspections of abattoirs revealed that the offal ban was being widely flouted.

In the mid-1990s, inspectors returned with tales of slaughterhouse staff claiming that they had been told to remove spinal cords from carcasses before cutting them up only two weeks beforehand. The revelation meant that cuts of meat contaminated by infected spinal cord had been finding their way into the human food chain for more than 10 years. Professor Collinge, who had recently joined the government's spongiform encephalopathy advisory committee, was dismayed. "It was clear the ban was a fiction and that it wasn't being policed," he says now. "But then, there were government ministers saying there was no

conceivable risk [to humans], so how could we expect abattoir workers to go to extraordinary lengths to dissect out spinal cords?"

The turning point in the transmission of vCJD to humans occurred in late 1995 (Almond et al., 1995) and quickly published (Liewelyn et al., April 1996).

In 1995, one month after ministers formed a new Meat Hygiene Service to police the abattoirs, news came that an 18-year-old, Stephen Churchill, had died of a CJD-like disease. He was later identified as the first confirmed victim of vCJD. Two more deaths that year were also attributed to vCJD. "Very rapidly it became apparent that what we feared was indeed beginning to happen," says Collinge.

In 2000, 28 people died from vCJD, the largest death toll recorded in one year, bringing the total at the time to 84. In each case, the disease was as harrowing as it was unstoppable. It began with a change in personality: happy, confident young people developed mood swings, depression, lapses in memory. Some were prescribed antidepressants by baffled GPs. Others ended up in psychiatric wards. Then more symptoms emerged. Pins and needles, limb pain. Neurologists eventually diagnosed degenerative brain disease. Later on, unsteadiness would set in, then speech difficulties, incontinence, jerky movements and progressive immobility. While sporadic CJD, an illness that strikes at random and usually affects the over-60s, takes a few months to kill, vCJD was infecting mostly young people and killing them more than a year later.

After 2000, however, the incidence of vCJD began to decline so the threat of latent infection, an experience from "cannibals" (Collinge et al., 2006).

From 2000, cases of vCJD began to tail off, but the threat is far from over and huge questions remain. Animal studies proved that less than a gram of BSE-contaminated material could infect cattle. In humans, the lethal dose remains unknown. Other unanswered questions are more worrying. Many scientists believe two more waves of vCJD are looming. Genetic tests on vCJD patients reveal that all of them, so far at least, belong to 40% of the population who have what is called the MM form of human prion protein. When BSE infects a human, it is this form of prion that is most susceptible to changing and causing vCJD. The remaining 60% of the population have different genetic

variants of human prion (known as MV or VV). When they are infected with BSE, tests suggest their prions will still eventually misfold into a form that causes vCJD, only the process will take much longer. The upshot is that two future waves of vCJD could strike in the next 10 to 50 years.

The fear of future waves of disease is real: it is backed up by animal tests and studies of cannibalistic behaviour in Papua New Guinea. Research published in the Lancet last year supports the idea. Collinge's group investigated an epidemic of brain disease called kuru, triggered by cannibalistic rituals that were only banned in Papua New Guinea in 1950. Like vCJD, the disease is caused by a rogue prion. They found that, even without a species barrier to jump, the infection could linger unnoticed in people for more than 50 years. Disturbingly, the first to succumb had the MM form of prion (Sample, 2007).

Accordig to Collinge et al., why the vCJD infection could linger unnoticed in people for more than 50 years ?

Incubation periods of infection with human prions can exceed 50 years.

In human infection with BSE prions, species-barrier effects, which are characteristic of cross-species transmission, would be expected to further increase the mean and range of incubation periods, compared with recycling of prions within species. These data should inform attempts to model variant CJD epidemiology (Collinge et al., 2006).

In brief, how was it "scientifically" found?

John Collinge at University College London, UK, and his colleagues went to Papua New Guinea to find the vCJD incubation. Most people who had kuru have already died, but the team ramped up existing disease monitoring in an attempt to find the last vestiges of the epidemic: those people who harboured the infectious prions for many years and are only just developing the disease. Working with local communities, they scoured isolated villages, which are typically located at mountainous altitudes above 2,000 metres, lost in dense, wet rainforest and often connected only by tracks. "It's arduous trekking," Collinge says.

From 1996 until mid-2004, the team found what they believe to be the final 11 remaining cases of kuru, which often manifests itself as problems with

balance and coordination. The researchers also collected people's life histories to piece together when they were likely to have been infected. Because the cannibalistic ritual had stopped by 1960, the team calculated the incubation time as the time between 1960 and the year that a patient first exhibited kuru symptoms. This suggested an incubation lasting between 34 and 41 years, although the researchers did not know exactly when, before 1960, a person was infected.

Collinge says that vCJD could well have an average incubation time of 30 years or longer (more than the average for kuru) because the prions are passing from cows to humans rather than from human to human. This species barrier is known to extend incubation times in animal tests. The people who have already succumbed to vCJD could have been those with the shortest incubation period, perhaps because they were particularly genetically susceptible, as other evidence has suggested. Mathematical models used to predict the size of the likely vCJD epidemic could now take these findings into account. "Most people seem to be thinking that we're over the worst of it," Collinge says. "We have to be cautious about assuming this disease is going away" (Pearson, 2006).

Conclusions

To sum up all the above, where - which cattle were fed meat-and-bone meal, the cause of all evil? When cases of BSE continued to rise, and by the end of 1989, nearly 10,000 cattle at more than 5,000 farms had tested positive. After all, it is not possible for anything like MBM to be not found in such a huge number of herds! Similarly, no MBM was fed for cows when in 1987/88 there 156 confirmed BSE cases in 145 cattle herds (with at least one confirmed case), and „hypothetical“ was concluded, that BSE has an origin in MBM feeding (Wilesmith et al., 1988).

However, there are still various speculations and hypotheses that British cows may have been fed certain MBM-containing kencentrates in addition to feedstuffs. However, such concentrates were not available to cows in pastures and yet suckler herds were also affected by BSE. According to well-known British statistics, according to DEFRA; the majority of BSE cases (ca 80%) occurred in dairy herds, however, ca 15% of cases have been reported in suckler herds !

More detailed informations on the initial occurrence of BSE in the UK, see Table 1 (BSE Inquiry, 2000b).

Table 1; Number of confirmed cases of BSE by production type, UK, to 20 March 1996 (BSE Inquiry, 2000b)

Production Type	England	Scotland	Wales	Great Britain	Northern Ireland	United Kingdom
Dairy/Mixed	124,207	4,301	12,019	140,527	1,331	141,858
Beef	12,568	3,296	2,995	18,859	378	19,237
Unknown	1,540	131	188	1,859	1	1,860
Total	138,315	7,728	15,202	161,245	1,710	162,955

Note, UK; Dairy/mixed – 87 %, Beef- 12 %, Unknown- 1%. However, in Scotland; Dairy/mixed cows – 55 %, and Beef cattle- 43 % !!!

A little over a third of the 92,000 or so herds in Great Britain had been affected by BSE (33,536)! Dairy herds were far more affected by BSE than beef herds, because dairy cattle were fed „protein concentrates“ - to a much greater extent than beef cattle. In contrast, these beef cattle were fed "energy concentrates," mostly as rolled barley (Thomas et al., 1988; Steen and Kilpatrick, 2000). From isolated BSE cases first identified retrospectively in May 1985 however, it is possible that the first cases of BSE occurred about 5 years earlier (BSE Inquiry, 2000a). It is believed that several million cattle with the BSE condition likely entered the food supply during the outbreak . So more than 35 years have passed since 1985 !There should have been thousands of dead people, but vCJD has already disappeared when the last case of vCJD was diagnosed in 2013.

However, in 2000, „scientifically“ was pointed out that; there is continued speculation about the likely number of cases of vCJD that will occur in Great Britain in the wake of the BSE epidemic in cattle. We show here that the current mortality data are consistent with between 63 and 136,000 cases among the population known to have a susceptible genotype (about 40% of the total population), with on average less than two cases of vCJD arising from the consumption of one infected bovine (Ghani et al., 2000). However, since 1996 and as of August 2013, a total only of 229 cases of vCJD cases have been identified from 11 countries: 177 from the UK and 52 cases elsewhere (ECDC, 2017).

It has gradually been scientifically proven that there is no need to be afraid of infection in CJD by blood transfusions or surgical instruments (2010). There is no need to be afraid of "infectious prions" (2012). And not at all from BSE / vCJD after beef consumption, which ended in 2013 (Hlásný, 2019).

So in the end, of all the BSE "hypothetical" stories, only the ban on feeding MBM to livestock in all EU countries remained, although nowhere and

never has MBM feeding been found in cattle (ruminants). So in the current economic crisis, if the EU governments are really interested in saving money, then a simple measure is offered ; to resume feeding the MBM to pigs and poultry. At the same time, this will not only help preserve rainforests in Brazil, but also reduce health risks (GM soybeans).

Note; In 2020, 98 percent of the soybean crops in Brazil were genetically modified (GM) to be glyphosate (Roundup) herbicide tolerant. By comparison, only 40 percent of soybean crops were genetically modified in 2005 (Proterra Foundation, 2021).

References

Alderman,G.- Cottrill, B.R. (1993); Energy and protein requirements of ruminants : an advisory manual prepared by the AFRC. *AGRIS*, 183 pages.

Almond,J.W. - Brown,P. - Gore, S. M. - Hofman, P. W.- Wientjens, R.M.- Ridley, H.F.- Baker, G..- Roberts, W.- Tyler, K.L. (1995); Creutzfeldt-Jakob disease and bovine spongiform encephalopathy: any connection? *BMJ*. 311, pp. 1415–1421. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC2544410/pdf/bmj00620-0039.pdf

ARC, 1965; The Nutrient Requirements of Farm Livestock. No. 2. Ruminants. *Agricultural Research Council, London*, 37 pages.

ARC 1980; The Nutrient Requirements of Farm Livestock. No. 2. Ruminants. Agricultural Research Council, 2nd ed. Commonwealth Agricultural Bureaux, *Farnham Royal, England*, 351 pages https://www.worldcat.org/title/nutrient-requirements-of-ruminant-livestock-technical-review/oclc/7087407

Armstrong, D. G.- Brookes, L. M. (1981); Protein requirements of ruminants : the ARC proposals. *Livestock library*, Australian Society of Animal Production http://www.livestocklibrary.com.au/handle/1234/19397

BBC, 2000; Donald Acheson: Whistleblower. *BBC*, 11 October, 2000, http://news.bbc.co.uk/2/hi/special_report/1999/06/99/bse_inquiry/372254.stm

Briggs,H. (2020); Amazon soya and beef exports 'linked to deforestation'. *BBC*, July 18, 2020 https://www.bbc.com/news/science-environment-53438680

Brown, P. (2001); Regular review: Bovine spongiform encephalopathy and variant Creutzfeldt-Jakob disease. *BMJ*, 322, pp. 841–844. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC1120011/

BSE Inquiry(2000a) ; Key conclusions, the cause of BSE. T*he BSE Inquiry,*Vol 1, London: Stationery Office

https://webarchive.nationalarchives.gov.uk/ukgwa/20060802142310/http://www.bseinquiry.gov.uk /
BSE Inquiry (2000b); Statistics, BSE incidence by herd, type of herd and breed. *The BSE Inquiry*, Vol 16, London: Stationery Office
Collinge,J.- Whitfield,J.- McKintosh,E.- Beck,J.- Mead,S.-D.J. Thomas- M.P. Alpers (2006); Kuru in the 21st century--an acquired human prion disease with very long incubation periods. *Lancet*, 367, pp. 2068- 74
https://pubmed.ncbi.nlm.nih.gov/16798390/
Ferhttps://www.worldcat.org/title/nutrient-requirements-of-farm-livestock-no-2-ruminants-summaries-of-estimated-requirements/oclc/223559575guson-Smith, M.A. (2001); BSE and variant CJD. Assumption that BSE originated from scrapie in sheep led to misjudgment. *BMJ*, 322; 1544 https://www.ncbi.nlm.nih.gov/pmc/articles/PMC1120584/
Ghani, A.C.- Ferguson, N.M.- Donnelly, C.A.- Anderson, R.M. (2000); Predicted vCJD mortality in Great Britain. *Nature*, 406, pp. 583-4.
https://www.nature.com/articles/35020688
Hlásný, J. (2019); Infectious diseases derived from meal and bone meal (MBM); epidemics that never was? *Výzkum v chovu skotu* (Rapotín), 61 (2), pp. 23-30.
Hlásný, J. (2020); Is magnesium deficiency the cause of neurodegeneration in animals and humans? 1st part; BSE „magnesium- ammonia“ alternative theory. *Výzkum v chovu skotu* (Rapotín), 62 (1), pp. 36- 47.
Leggett,M.M.- Dukes, J.- Pirie, H.M. (1990); A spongiform encephalopathy in a cat. Vet.Rec., 127 (24), pp. 586- 588
https://pubmed.ncbi.nlm.nih.gov/2075687/
Liewelyn,C.A.-Hewitt, P.E.- Knight, R.S.- Amar, K- Cousens, S.- Mackenzie, J.- Will,R.G. (2004); Possible transmission of variant Creutzfeldt-Jakob disease by blood transfusion. *Lancet*, 363, pp. 417- 421.
https://www.ncbi.nlm.nih.gov/pubmed/14962520
OIE, 2018; Bovine spongiform encephalopathy. OIE. World Organisation for Animal Health, 8 June, 2018 https://www.oie.int/en/disease/bovine-spongiform-encephalopathy/
Pearson, H, (2006); CJD-related disease can incubate for 50 years. *Nature*, published online 23 June, 2006
https://www.nature.com/news/2006/060619/full/news060619-12.html
Proterra Foundation (2021); The non-GM production: how volatility is killing the supply chain in Brazil, February 16, 2021.
(https://www.proterrafoundation.org/news/the-non-gm-production-how-volatility-is-killing-the-supply-chain-in-brazil/)

Reuters, 2021; European Supermarkets Stop Selling Brazil Beef Over Deforestation Links. *US News*, 15 December , 2021
https://money.usnews.com/investing/news/articles/2021-12-15/european-supermarkets-stop-selling-brazil-beef-over-deforestation-links
Sample,I.; Should we still be worried? *The Guardian*, 10 January , 2007
https://www.theguardian.com/society/2007/jan/10/health.bse
Steen, R.W.J. - Kilpatrick, D.J. (2000) ; The effects of the ratio of grass silage to concentrates in the diet and restricted dry matter intake on the performance and carcass composition of beef cattle. *Livestock Production Science*, 62, pp. 181–192 https://citeseerx.ist.psu.edu/viewdoc/download?doi=10.1.1.1038.9854&rep=rep1&type=pdf
Thomas, D., Gibbs, B.G., Beever, D.E., Thurnham, B.R., (1988); The effect of date of cut and barley substitution on gain and on the efficiency of utilization of grass silage by growing cattle. *Br. J. Nutr*. 60, pp. 297–306 https://pubmed.ncbi.nlm.nih.gov/3196672/
Whitaker,D.A. - Kelly, J.M.- Smith, E.J. (1985); Hypomagnesaemia in increase. *Vet Rec.*,116 (16), pp.451- 52.
https://www.ncbi.nlm.nih.gov/pubmed/4002580
Whitaker, D.A.- Kelly, J.M. -Eayers, H.F. (1993); Incidence of hypomagnesaemia. *Vet Rec.*,132(26), p. 662.
Wilesmith, J.W.- Wells,G.A.- Cranwell, M.P,- Ryan,J.B. (1988); Bovine spongiform encephalopathy: epidemiological studies. *Vet Rec.*, 123(25), pp. 638-644. https://www.ncbi.nlm.nih.gov/pubmed/3218047
Wilesmith,J.W.- Ryan,J.B.- Atkinson, M.J. (1991); Bovine spongiform encephalopathy: epidemiological studies on the origin. *Veter Rec*., 128 (9), pp. 199- 203 https://pubmed.ncbi.nlm.nih.gov/1823120/
Wilesmith,J.W. (2000 ; Statement No 91 Dr John Wilesmith (scheduled to give oral evidence 22/06/98). T*he BSE Inquiry*, London: Stationery Office
Wilson, P.N.- Brigstocket, D.A. (1981); Improved feeding of cattle and sheep. *The BSE Inquiry* (2000); 15 pages.
Winter, M, H.- Aldridge,B.M.- Scott,P.R.- Clarke, M. (1989); Occurrence of 14 cases of bovine spongiform encephalopathy in a closed dairy herd. Brit.Vet. J. 145 (2), pp. 191-194. https://www.ncbi.nlm.nih.gov/pubmed/2713640)

HLÁSNÝ, J. (2022); Computer diagnostics (professional journal) on the origin of bovine spongiform encephalopathy (BSE) and experimental diagnostics that exclude meat and bone meal (MBM) as the cause of BSE origin (scientific journal).Výzkum v chovu skotu 64 (2), pp. 34- 45

https://www.vuchs.cz/main/bulletin/vyzkum-v-chovu-skotu-2-2022/

When looking for evidence that MBM is the cause of "BSE infection", it is advantageous to use opinions from "reputable" proponents of infectious prion theory. For example, a study entitled: "Risk Analysis; BSE Risk from Importation of Designated Ruminants and Ruminant Products from Canada into the United States". This is a study from the end of October 2003 that discusses the risks to the United States following the finding of a "single" BSE-positive cow in Canada. In this study, it is stated, "The primary source of BSE infection is commercial feed contaminated with an infected agent. Scientific evidence (WILESMITH et al.; 1988, 1991, 1992) shows that feed contamination occurs by incorporation (mixing) of components that contain ruminant protein from infected animals… Only oral administration of contaminated feed with abnormal BSE protein is a documented BSE transmission route under normal conditions (WILESMITH et al.; 1988, 1991, 1992), although other possibilities have been considered…. ". So even twice, American experts have drawn attention to publications that allegedly provide "scientific evidence" that MKM is- was the cause of BSE in Britain.

Ten years later French scientists have also pointed to infection during changes in the rendering process, according to Wilesmith et al., 1991 (Ducrot et al., 2013) . Due to certain doubts in Czech veterinary practice, we analyzed the three journals- theories in more detail (Hlásný, 2004a). The conclusion was that MBM is not listed as the causative agent of BSE, you just need to read the publications, which the above-mentioned experts probably did not do and just "copied" the information.

a/ Bovine spongiform encephalopathy; epidemiological studies (Wilesmith et al., 1988).

In Britain, much of the alarmism about Mad Cow disease was never justified scientifically. It was pure, math-model-driven science fiction, just like Global Warming. But it was pushed very vigorously by the British science

establishment, which has never confessed to its errors, and is therefore likely to make the same ones again. In politicized science, public hysteria actually builds careers; in real science, it tends to ruin careers. Right across the Channel in Britain, farmers were required by law to destroy and bury hundreds of thousands of sheep and cows. It was an economic disaster, and all because of wildly alarmist science (Lewis, 2007).

In December 1988, Wilesmith et al. published their initial "simulations" on the cause of BSE in the journal "Veterinary Record". Computer simulations even concluded that calves were most affected by "eating" meat and bone meal (MBM); this was later "simulated" that they were calves at the age of 2 weeks. However, in my opinion, these calves would have died 100% after such a diet - they had become an incurable, neonatal diarrhea. Unfortunately, this lie, perhaps not a hundred times but more than a thousand times, has been considered true to this day. Today, there is only one address on the Internet for what was at the time (only the abstract is available).

Abstract

This study, initiated in June 1987, describes the epidemiology of bovine spongiform encephalopathy (BSE), a recently described novel neurological disease of domestic cattle first identified in Great Britain in November 1986. Records suggested that the earliest suspected cases occurred in April 1985. There was variability in the presenting signs and the disease course, but the majority of cases developed behavioural disorders, gait ataxia, paresis and loss of bodyweight; pruritus was not a predominant sign. The form of the epidemic was typical of an extended common source in which all affected animals were index cases. The use of therapeutic or agricultural chemicals on affected farms presented no common factors. Specific genetic analyses eliminated BSE from being exclusively determined by simple mendelian inheritance. Neither was there any evidence that it was introduced into Great Britain by imported cattle or semen. The study supports previous evidence of aetiological similarities between BSE and scrapie of sheep. The findings were consistent with exposure of cattle to a scrapie-like agent, via cattle feedstuffs containing ruminant-derived protein. It is suggested that exposure began in 1981/82 and that the majority of affected animals became infected in calfhood.

Methods; Analyses of data

A computer simulation model was constructed, on the hypothesis that BSE is caused by a transmissible agent, to examine the time of onset and duration of exposure, the incubation period distribution and age classes of animals exposed.

The model consisted of a large population of calves, young stock and adults; the latter were subjected to age specific culling rates obtained from previous studies of dairy herds (J.W.Wilesmith and J.B.M.Ryan, unpublished observations). Values for the exposure parameters and incubation period were imposed on the model. The incubation period was assumed to have a log normal distribution, and to exhibit no variation with age at first exposure. The age specific incidences of BSE in herds with confirmed cases during 1987 were used to asses the validity of the exposure and incubation period parameters. The simulation was also used to predict changes in age specific incidences in future years.

Results

The frequency of the presenting clinical recorded for 156 confirmed cases; The most common history given by the herdsmen was "nervousness" (Fig 1). The distribution of 145 herds, with at least one confirmed case, by their cattle purchasing policy and the presence of sheep on the farm since 1980 showed that in 15 per cent of herds no cattle had been purchased and 20 per cent of herds had had no contact with sheep (Table 5). The use of a computer based simulation model indicated that the values of the age specific incidences observed in 1987 were consistent with the following features. First, both calves and adults (over two years old) have been exposed, but the risk for calves was 30 times that for adults; secondly, exposure of the cattle population commenced in the winter of 1981/1982 and continued to at least the end of 1984 and, thirdly, an incubation period with the range 2-5 years to at least eight years and a log normal distribution. The maximum incubation period that could have been observed in 1987 was six years. Further epidemiological data for 1988 and subsequent years is needed to determine whether exposure continued after 1984.

Discussion

The results of the present study preclude the transmission of the scrapie agent from sheep to cattle via direct or indirect contact on affected farms because of the form of the epidemic and the absence of sheep on 20 per cent of farms with BSE. The results of the study do, however, lead inevitably to the conclusion that cattle have been exposed to a transmissible agent via feedstuffs.

Summary

This study initiated in June 1987, describes the epidemiology of BSE, described novel neurological disease of domestic cattle first identified in Great

Britain in November 1986... The study supports previous evidence of aetiological similarities between BSE and scrapie of sheep. The findings were consistent with exposure of cattle to a scrapie-like agent via cattle feedstuffs containing ruminant-derived protein. It is suggested that exposure began in 1981/ 82 and that the majority of affected animals became infected in calfhood.

Conclusion

So now specifically about the first results of the work of authors where they do not mention any computer simulation at all in both the abstract and the summary. So, if one does not notice that they use the word "suggested" in the abstract and in the summary, then the reader has no doubt that the "newborn calves" were actually fed by MBM. Well, in short, "professional lie" (see professional journal) is still presented as "scientific truth".

The abstract contains only meaningless information, when the data from the results should be presented there. So if someone doesn't get a job and doesn't read an article in "The Veterinary Record," they don't find that;

there is no infection when in 145 bovine herds, in the vast majority only one confirmed case was detected

the infection should have nothing to do with sheep, as 20% cattle could have no contact with sheep

a computer simulation model was constructed based on the hypothesis that BSE is caused by a transmissible agent, so that it was not found at all that the calves were fed MBM (animal protein)

these are just some "computer simulations" where no one has ever seen or known the feeding of MBM calves and at the same time this "suggestive hypothesis" is still recognized in the world,

In short, it is unbelievable why this "BSE proposal" is still valid, when in the age of the Internet it is very easy to find relevant publications in a matter of seconds and evaluate them with common sense. In such "fake news", however, the authors abstractly "cover up" by saying that they are mere "suggestions - assumptions".

b/ Bovine spongiform encephalopathy: epidemiological studies on the origin (Wilesmith et al., 1991)

Abstract

The results of further epidemiological studies of bovine spongiform encephalopathy (BSE) support the previous findings that the onset of exposure of the cattle population to a scrapie-like agent, sufficient to result in clinical disease, occurred in 1981/82. The onset of this exposure was related to the cessation, in all but two rendering plants, of the hydrocarbon solvent extraction of fat from meat and bone meal. A further possible explanation, related to the geographical variation in the reprocessing of greaves to produce meat and bone meal, was identified for the geographical variation in the incidence of BSE.

Conclusion

production of MBM." However, this sentence from the abstract of the publication and other text do not prove anything in terms of MBM infectivity; as follows from the following designation of all "pictures-graphs" and the only table in the study results: Fig.1: Occurrence of BSE by age in affected herds of cows in 1987 and 1988 Fig.2: Maximum temperatures reached at continuous (continuous) and at Fig. 3: Ratio - proportions of produced MBM using continuous rendering processing in the period 1971-1988 (based on comparison with production in 1988) Fig.4: Ratio - proportions of produced MBM using solvent extraction from 1964 to 1988 (Hlásný, 2004a).According to The BSE Inquiry (October 2000); the theory that BSE resulted from changes in rendering methods has no validity. Rendering methods have never been capable of completely inactivating TSEs. Similarly, scrapie has mysteriously chosen the United Kingdom as its only geographical site and the early 1980s as its only historical occurrence to appear in cattle, see BSE Inquiry (2000). The origin of BSE can only be inferred in this publication from the title of the article. So this publication has nothing to do with MBM, as the cause of BSE.

So this publication has nothing to do with MBM, as the cause of BSE. Even in this publication, nowhere does it appear to be; "Found-proven-found ..." that MBM has been identified as a source of BSE infection. Again, however, there is one sentence as "convicting" of an MBM infection, namely that; "Geographical variability in the occurrence of BSE has been identified as another possible explanation in relation to geographical variability in the 'processing of greaves' in the production of MBM." However, this sentence from the abstract of the publication and other text do not prove anything in terms of MBM infectivity; as follows from the lowing designation of all "pictures-graphs" and the only table in the study results: Fig. 1: Occurrence of BSE by age in affected herds of cows in 1987 and 1988 Fig. 2: Maximum temperatures reached at continuous

(continuous) and at Fig. 3: Ratio – proportions of produced MBM using continuous rendering processing in the period 1971–1988 (based on comparison with production in 1988) Fig. 4: Ratio – proportions of produced MBM using solvent extraction from 1964 to 1988 (Hlásný, 2004).

c/ Bovine spongiform encephalopathy; case- control studies of calf feeding practices and meat- and- bone meal inclusion in proprietary concentrates (Wilesmith et al., 1992).

Abstract

Following the identification of meat and bonemeal as the most likely source of exposure for the occurrence of bovine spongiform encephalopathy (BSE) in Great Britain case-control studies were initiated to investigate this hypothesis. These involved a comparison of the consumption of specific proprietary calf feedstuffs, and whether or not meat and bonemeal had been included, between animals born in 1983-84 in BSE-unaffected herds and confirmed cases of BSE also born in 1983-84. The feeding of proprietary concentrates containing meat and bonemeal was found to be a statistically significant risk factor for the occurrence of BSE. These studies therefore support the initial hypothesis that BSE occurred as a result of exposure to a scrapie-like agent via meat and bonemeal.

Conclusions

Mr. Wilesmith and others began to examine the use of MBM and tallow in cattle feed. For example he and Mr Keith Meldrum (Deputy CVO) met with Dr Laurson-Jones, a veterinary colleague in the feed industry in early January 1988. At this time he received from Mr Gallehawk the data which he had requested on the composition of livestock rations. This showed that between 1979 and 1987, there had been no increase or new use of MBM in cattle feed (Wilesmith, 2000). So this publication has nothing to do with MBM, as the cause of BSE

Ben GILL (The BSE Inquiry / Statement No 47, April, 1998) ; former chairman of the Livestock and Wool Committee of the National Farmers' Union (NFU) , he says: " Feed compounds used for feeding cattle, farmers may buy compound feed from feed producers. However, the absence of ingredient listing meant that farmers buying compound feedstffs would not know whether or not the feed included MBM. For example, protein could amongst others be generated by soya bean meal, or from processed MBM. 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 MBM.

Feeding MBM was only scientifically recommended to feed high-yielding cows (cattle) due to the supply of non-degradable protein. It is not known from any literature that MBM has been fed to ruminants in practice. However, MBM shows a rendering odor, so ruminants refuse to accept feed containing MBM. However, if MBM is added to the feed, then it is necessary to create a certain habit and gradually increase the content of MBM in the feed (McDonald et al., 1988). So the packaging of the feed mixture-concentrate must be marked whether the MBM contains feed.

II. Experimental dairy cow nutrition and exclusion of BSE infectious origin, without feeding meat-and-bone meal (Dewhurst et al., 2000; Moorby et al., 2000)

BSE researchers usually cite only studies where there is only a "suspicion" of a link between MBM, BSE (and vCJD). However, none of them cites a publication that is completely different from all the others; because it describes BSE in cows that "accidentally" appeared during a nutritional experiment without the use of MBM (Dewhurst et al., 2000; Moorby et al., 2000). The experiment is described as applying three types of feed ration based on first cut ryegrass silage (ad libitum) to 47 cows during the last six weeks of gestation (dry period) as follows: a / ryegrass silage and barley straw in a ratio of 60 : 40 (GSBS group) , b / ryegrass silage alone (GS group) c / the same silage ad libitum with the addition of 0.5 kg of protein meal (gluten meal = prairie meal) per piece and day (GSPM group).

Thus, in their study, the authors refer to a nutritional experiment that was previously "closed" and published in the mentioned scientific journal (manuscript delivered in October 1999; published in August 2000), rather than to a follow-up study in a professional journal. "Veterinary Record" (Moorby et al., 2000a), published on October 7, 2000. Thus, it appears that only after the manuscript of both articles was submitted (September, 1999) were the BSE cases confirmed in the laboratory. So only "additionally", in August 2000, they completed and supplemented the original nutrition study with findings on the detection and confirmation of BSE in six of the 47 experimental cows. This also corresponds to the fact that both articles in the mentioned scientific journal do not mention at all that clinical signs of BSE were observed during the experiment. By providing more detailed data on cow metabolism in one (USA) and data on BSE in another journal (UK) - this seems to have contributed to the occurrence and finding of BSE, with very accurately evaluated indicators of protein metabolism- without the citation in the world literature.

After calving, all cows received ryegrass silage ad libitum with the gradual addition of a standard feed mixture ("concentrate"). Blood samples were taken weekly before calving and then in the 1st, 3rd, 5th, 7th, 13th, 17th and 21st weeks after calving. In the period after the last blood samples were taken (ie 27 weeks after the start of the experiment); A total of six cows began to show clinical signs of BSE, which was later confirmed histologically. There were three cows (GSBS group), one cow was from the group of cows fed a silage monodiet (GS) during the dry season and two BSE-affected cows were from the GSPM group. Five of these cows were slaughtered within one to four months after the last sampling, the sixth cow only in 21 months. The authors point out that by September 2000, no next case of BSE had occurred.

As this is a very detailed nutritional experiment, it is appropriate to compare the results of the experiment with how the origin of BSE is explained using an alternative "magnesium-ammonia theory" (Hlásný, 2001). Returning to the first publication in a scientific journal (Dewhurst et al., 2000), it states at the outset that the aim of this work is to determine the short-term and long-term effects of changes in energy and protein intake in cows in the last six weeks of pregnancy. for dry matter intake (DM), physical condition and milk production.

The already common supply of Mg in the concentrate is also observed here. The second publication (Moorby et al., 2000) states that the aim of the experiment (common in Western European conditions) was to determine the effect of changes in protein and energy intake in dairy cows during the dry period; plasma hormone and metabolite levels during dry standing and during the first 21 weeks of subsequent lactation. In the section "Material and methods" of both scientific publications, as in the professional journal Veterinary record; is described as the same feed ration in three different groups of cows, during the last six weeks before calving and during the 22-week lactation period (Hlásný, 2004).

More detailed results from the nutritional experiment

So now in more detail, according to the Scientific Journal of Dairy Science. This is basically the only study in more than 30 years that describes the effects of nutrition on BSE in more detail. From the whole text of two articles in a scientific journal (see text below), certain passages were selected, which were subsequently interpreted in 2004 in the journal "Výzkum v chovu skotu" (Hlásný, 2004);

1. Regarding the more detailed indicators for ryegrass silage, this can be characterized by the fact that it was a younger to medium-old forage (see the

CP, NDF, and ADFcontent) and the corresponded content of crude protein (CP) , it is indicative of this that it was not a more intensively nitrogen-fertilized grassland. This follows from the following table:

Table 1. Chemical analysis of the silages (toluene DM, %)

	Dry period	Lactaton
Dry matter	20,36	20,77
Crude protein (N x 6,25)	14,9	14,9
Soluble N	1,61	1,50
Ammonia N	0,20	0,22
NDF	50,0	51,8
ADF	29,9	32,0
Watersol. carbohydr.	0,74	0,68
Ph	3,98	3,85
Lactic acid	6,70	7,57
Acetic acid	2,28	2,05
n- butyric acid	0,06	0,20

2. During the lactation period, all cows were fed, in addition to ryegrass silage, a compound feed ("concentrate") with the following composition;

Table 2. Composition of the concentrates (% of DM)

	Wk 1-4	WK 5-22 of lactation
Barley	2,4	0
Corn	6,66	0
Wheat	28,14	42,30
Corn germ	10,94	10,13
Corn gluten meal	14,78	10,36
Field beans	3,31	0
Hipro- soybean meal	22,43	25,18
Molasses	5,66	6,39
Dairy fat	1,12	1,13
DCF	1,12	1,13
Limestone	1,46	1,47
Calcined magnezite	0,76	0,79
Salt	0,86	0,84
Mineral vit.premix	0,28	0,28

Chemical analysis of the concentrates (%)

DM	88,0	88,3
Crude protein	22,3	22,6
Soluble N	0,61	0,47
Starch	36,4	38,4
Water sol.carbohydr	9,0	10,0

Note mineral- vitamine content:

Calcined Mg; MgO (90 to 95%), CaO (1.5 to 2.5%); SiO2 (1 to 2%). 3 Mineral and vitamin premix supplied (on a concentrate DM basis): 14,200 IU of vitamin A/kg, 2900 IU of vitamin D3/kg, 22.7 IU of vitamin E/kg, 4.5 mg/kg of Co, 34 mg/kg of Cu, 57 mg/kg of Fe, 57 mg/kg of I, 137 mg/kg of Mn, 0.23 mg/kg of Se, and 136 mg/kg of Zn.

It follows from the above that, as far as the magnesium (Mg) content is concerned, it was present in the concentrate at a level of at least 0.5% at the stated dosage of "calcined magnesite" (mainly MgO). However, this is only an "estimate" because the content of minerals (magnesium v) in the feed used was not reported in this nutritional experiment. It is worth noting, however, that the formulation of this concentrate has been formulated to bring it closer to the common "reality of dairy cow nutrition in Western Europe". So the relatively high concentration of Mg in the "concentrates" used in cows feed ration (FR). was probably already commonly for years recommended …

3. The aim of the experiment was to significantly differentiate nitrogen intake in the dry period, which is shown in the following table;

Table 3. Intake; DM, energy, nitrogen and N balance in the dry period

	GSBS	GS	GSPM
DM intake (kg)	9,9	10,7	10,9
ME(MJ/day)	86,10	114,10	118,80
Crude proten intake (kg)	1,09	1,63	1,96
N intake (g)	174,0	261,0	314,0
N output (g) and rumen analyses			
Feces	64,0	76,0	86,0
Urine	101,0	151,0	178,0
N balance	9,0	34,0	50,0
Ammonia intake in rumen (mg/l)	84,0	111,0	128,0
Rumen pH	6,61	6,61	6,54

It follows that there was indeed a significant "gradation" in the intake of nitrogenous substances (proteins), as the group of cows with the addition of barley straw received 11% of protein; group of cows with silage monodiet 15.2% protein; group of dairy cows with the addition of 0.5 kg of protein flour 18% protein in dry matter FR. This was also related to the increasing intake of metabolizable energy (ME). The increasing concentration of ammonia in the rumen (84, 111, 128 mg / l, respectively) also corresponded to the increasing protein intake. So when it comes to nitrogen intake; Only FR (GSBS) with the addition of barley straw (40% in FR dry matter) met the physiological needs,

but even here a slightly higher concentration of ammonia in the rumen was found. The remaining 60% was ryegrass silage, which probably led to a more pronounced Mg deficiency in this group. Magnesium intake was higher in the protein meal (GSPM) group; however, too high a concentration of nitrogen (and probably potassium) in the FR reduced the utilization of Mg.

4. Other data show, however, that even during the lactation period, already in the KD of all experimental cows, there was a high protein content, well above the standard of nutrient requirements (see; NRC, 2001), as shown in the following overview;

Table 4. Influence of different feed rations (dry period) on some indicators (kg) from the 2nd to the 22nd week of lactation

	GSBS	**GS**	**GSPM**
Silage DM intake	9,96	10,29	9,85
Concen. DM intake	7,31	7,34	7,34
Total DM intake	17,27	17,63	17,19

Cow BW (kg) and milk production

Wk 2. lactation	559	584	610
Wk 22. lactation	596	599	612
Milk production (kg/day)	26,1	26,7	26,7

Milk composition (%)

Fat	4,00	4,05	4,02
Protein	3,07	3,08	3,04
Lactose	4,65	4,67	4,75

5. However, both publications of the scientific journal do not state the content of nitrogenous substances (proteins) in the dry matter (DM) of the feed ration (FR). Using the data in Table 4 as well as the following data, the nitrogen balance during lactation can be "estimated" as follows;

Table 5. Intake, nitrogen output and milk production (kg / day); 8th to 18th week of lactation.

	Wk 8			Wk 18		
	GSBS	**GS**	**GSPM**	**GSBS**	**GS**	**GSPM**
N intake (g)	548	569	553	485	485	490
Milk	137	139	143	116	118	111
Feces	150	143	147	133	123	132
Urine	232	241	245	227	217	241
N balance	29	46	19	10	27	5
Milk yield, kg/day	30,1	30,0	31,0	23,5	23,8	23,4

6. Very high levels of blood urea, as evidenced by the following values, are evidence of high protein intake;

Table 6. Average values of urea in blood plasma
Urea (mM)

	GSBS	**GS**	**GSPM**
6, ,5, and 4 Wbefore calving	29,3	33,0	36,4
3, 2, and 1 Wk before calving	30,6	33,5	36,3
1 ,3, and 5 Wk of early lactation	37,6	38,2	39,9
7,13, 17, and 21 Wk of lactation	41,0	41,2	42,9

Physiologic of blood urea nitrogen values, dairy cow early lactation; ca 5 mmol/l = 14 mg/dl. So values about 36.4 mg/ dl = 13 mmol/l (tab. 6). Subclinical ketoses did not appear throughout the experiment (see physiological blood glucose levels), but hyperammonemia may have occurred, especially during lactation. This also follows from the data on the higher concentration of ammonia in the rumen (Table 3) already in the dry period (84, 111, 128 mg / l), which corresponds to the values (GSBS, GS and GSPM); 46.5 mmol / l; 61.5 mmol / l and 70.9 mmol / l, respectively. Due to the detected urea levels, the values of ammonia in the rumen were probably even higher during lactation.

7. However, both publications of the scientific journal do not state the content of nitrogenous substances (proteins) in the dry matter of the FR. Using the data in Table 4 as well as the following data, the nitrogen balance during lactation can be "estimated" in the dry matter (%) as follows;

Table 7. Crude protein in feed ration DM intake

	Wk 8. of lactation			WK 18. of lactation		
	GSBS	**GS**	**GSPM**	**GSBS**	**GS**	**GSPM**
CP intake. kg/day	3,42	3,56	3,46	3,03	3,03	3,06
CP (%) in DM feed ration	19,7	20,5	19,9	17,4	17,4	17,6

Thus, the content of CP in the dry matter of the feed ration was around 20% in the first weeks of lactation. According to the then valid standard (NRC, 1989); however, only in the first weeks of lactation it was recommended- "standardized" 19% of proteins in DM. Dry matter intake averaged around 17.4 kg (17.19 to 17.63) - in the period from the 2nd week to the 22nd week of lactation (Table 4). If the stated average value in dry matter intake is used to calculate the nitrogen concentration (CP) and if the nitrogen intake is converted to nitrogenous substances (N x 6.25), then the above data can be obtained.

According to the currently valid standard (NRC, 2001) it is only 15.9% of CP in FR dry matter in the first weeks after calving. In the following period at the milk yield of about 25 kg of milk (kg /day), it should be only about 14% protein. It follows that the protein intake was quite high not only in the last 6 weeks before calving (GS and GSPM group), but also throughout lactation. During the lactation period, about 10 kg of silage dry matter was fed (Table 4), which at about 20% of silage dry matter (Table 1) represented about 50 kg of silage/ day in cows about 600 kg.

As far as ryegrass (English or Italian) is concerned, it is known that subclinical hypomagnesaemia should be considered differentially when feeding it at higher doses (Smith, 1996). This is because ryegrass has the highest K / (Ca + Mg) quotient values, with a high correlation between potassium content and NL content (Hlásný, 1989; Hlásný, 1990). It is also related to his ability; respond maximally by mass yield at higher NPK fertilization intensity. Similar recommendations are known from Cullough (1994), which was later recalled (Hlásný, 1996). However, according to the NRC (1989) high protein level it was recommended only during first lactation after calving. So above mentioned recommendation were "overdosed" in dairy cow practice.

The research during 1990s resulted to decrease of protein content in dairy cow rations (CP; early lactation, especially); see the comparison of the NRC (1989 and 2001);

Table 8

Dairy cow: 600-680 kg body weight				
	Lactation			Early lactation
Milk yield (kg/day)	35	45	55	35
Degradable protein – "DP" (%):				
NRC,1989	9.7	10.4	10.4	9.7
NRC,2001	9.7	9.8	9.8	10.3
Undegradable protein- "UDP" (%):				
NRC,1989	5.7	6.0	6.3	7.2
NRC,2001	5.5	6.2	6.9	5.6
Crude protein – "CP"- (%):				
NRC,1989	16.0	17.0	17.5	**19.0**
NRC,2001	15.2	16.0	16.7	**15.9**

III. Other observed results in BSE detection and interpretation of the reported articles (Moorby et al., 2000a)

Based on the detailed data on protein metabolism, it can be realistically concluded that this was a long-term excess of protein in cows, with insufficient use of Mg, due to hyperammonemia. This should then be in line with "magnesium- ammonia theory" - which has been described as a possible mechanism for BSE origin (Hlásný, 2001), on the basis of "inspiration" from the described publication in The Veterinary Record (Moorby et al., 2000a). Unfortunately, it is not mentioned anywhere; although hundreds of publications have been cited; however, dealing only with "hypotheses" about the cause of BSE, in relation to the "infectivity" of meat-and-bone meal (MBM) originating in Britain.

Returning to the BSE study on the incidence of BSE, no significant differences in dry matter intake were found between the BSE positive and BSE negative groups during the dry period. Feed intake gradually decreased with continuing pregnancy, by approximately 0.5 kg of DM per week. After calving, the dry matter intake of FR increased in both groups with the highest intake in the period around 11 weeks after calving. At the end of the 12th week after calving, the dose of concentrate was reduced (and thus the silage intake increased).

However, the data show that almost 13% of the cows involved in the experiment affected BSE. However, this percentage is quite high because, under normal circumstances, there was a BSE epidemic in Britain; detected a maximum of about 3% of positive cows, out of the total number of cows bred in the herd. It is known from the literature that the formation of "insoluble" magnesium ammonium phosphate in the rumen already occurs at a rumen

ammonia concentration of less than 40 mmol / l - at a rumen pH in the range of 6.2-7.2 (Axford et al., 1982), with ammonia concentrations of 30 to 70 mmol / l being common in the intake of excess protein from young grassland (Martens and Rayssiguier, 1980). It is known from the literature that the formation of "insoluble" magnesium ammonium phosphate in the rumen already occurs at a rumen ammonia concentration of less than 40 mmol / l - at a rumen pH in the range of 6.2-7.2 (Axford et al., 1982), with ammonia concentrations of 30 to 70 mmol / l being common in the intake of excess protein from young grassland (Martens et Rayssiguier, 1980).

From the full text of two articles by J. Dairy Sci. and Vet.Rec. (see text above) certain passages were selected, which were already published-interpreted in 2004 in the journal "Výzkum v chovu skotu" (Hlásný, 2004¨).

Conclusions

What was important about this attempt "inadvertently" (typical of; trial and error)?

that neurodegeneration in cows is not caused by an MBM infection (BSE) but that it is a "metabolic disorder" in some animals susceptible to this disorder

that the incubation period of "infectious" transmission of neurodegeneration is not about 5 years, but only about 5 months

it is pointed out that this is the BSE creation of dairy cows, which were fed common in Western Europe during the 1990s (according to the NRC, 1989)

so higher protein intake was common at the time (compared to the later NRC, 2001)

Mg-supplementation was already common at that time (see the inclusion of MgO in the recipe of concentrates)

the nutrition of the dairy cows in the experiment complied with the former standards (NRC, 1989), but in practice, since about 1993, recommendations for lower protein intake (BSE decrease) have been applied in Britain compared to the EU countries (Alderman, 1993).

in contrast to that British in the EU states, cows were still given a high protein intake not only during the early lactation period, but throughout the lactation (McCullough, 1994).

Consumers in Europe should be informed by all media that the BSE infectious hypothesis based on MBM feeding has been wrong from the outset. Nowhere and never was it found that cows (cattle) were fed MBM! Possible experiments were not and could not be performed, because simply put - MBM ruminants do not eat! MBM should also be produced from ruminant tissues.

Red meat (ruminants) contains the most L-carnitine of all foods (feeds) needed for heart function... (Hlásný et Golda, 1997). MBM contains the most needed, organically bound phosphorus of all components.

References

ALDERMAN, G. – COTTRILL, B. R. (1993): Energy and protein requirements of ruminants: an advisory manual prepared by the AFRC. AGRIS, 183 p., https://agris.fao.org/agris-search/search. do?recordID=GB9406276

DEWHURST, R.J.- MOORBY, J.M.- DHANOA, M.S.- EVANS, R.T.- FISHER, W.J. (2000); 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(8); 1782- 1794. https://www.ncbi.nlm.nih.gov/pubmed/10984155

DUCROT, CH. – MATHILDE, P. – CALAVAS, D. (2013): BSE risk and the use of meat and bone meal in the feed industry: perspectives in the context of relaxing control measures. Nat Sci Soc., 21(12), pp. 3– 12, https://www.cairn.info/revue-natures-sciences-societes-2013-1-page-3.htm

HLÁSNÝ, J. (1989): Content of macrominerals, nitrates, and soluble sugars in feedstuffs. Vet. Med. (Prague), 34(9), pp. 567–576.

HLÁSNÝ, J. (1990): Evaluation of some relationships between macrominerals and nitrogen compounds in forages. Agrochémia (Bratislava), 30(1), pp. 28–32.

HLÁSNÝ, J. (1996): Typové krmné dávky u vysokoproduktivních dojnic. Výzkum v chovu skotu (Rapotín), 38(2), pp. 25–28.

HLÁSNÝ, J. – GOLDA, J. (1997): Biologická hodnota masa z hlediska obsahu karnitinu, jedné z nepostradatelných živin ve výživ˘e lidí. Výzkum v chovu skotu (Rapotín), 39(1), pp. 22–26.

HLÁSNÝ,J. (2001): Nervous diseases and connections with nutrition in ruminants. Výzkum v chovu skotu (Rapotín), 43(1), pp. 13–29, http://www.bse-expert. cz/pdf/BSE_publikace.pdf

HLÁSNÝ, J. (2004): Je skutecne masokostní moucka puvodcem „BSE infekce“? Casopis komory veterinárních lékaru CR˘ (Brno), 11(5), pp. 13–16, http:// www.bse-expert.cz/pdf/BSE_publikace.pdf

HLÁSNÝ, J. (2004a): Zapomínaný experiment s výskytem BSE p˘ri nadbytku bílkovin u dojených krav. Výzkum v chovu skotu (Rapotín), 46(2), pp. 25–35, http:// www.bse-expert.cz/pdf/BSE_publikace.pdf

LEWIS, J. (2007): Global Warming’ as Pathological Science. American Thinker. November 2, 2007,

https://www.americanthinker.com/articles/2007/11/global_warming_as_pathological.html
McCULLOUGH, M. E. (1994): Total mixed rations and supercows. Hoard´s Dairyman, 63 pp.
McDONALD, P. – EDWARDS, R. A. – GREENHALGH, J. F. D. (1988): Animal Nutrition. London, 543 pp.
MOORBY,J.M.- DEWHURST,R.J.- TWEED,J.K.- DHANOA, M.S.- BECK, N.F. (2000); Effects of altering the energy and protein supply to dairy cows during the dry period. 2. Metabolic and hormonal responses. J Dairy Sci., 83(8); 1795-1805. https://www.ncbi.nlm.nih.gov/pubmed/10984156
MOORBY,J.M.- DHANOA, M.S.- AUSTIN,A.R. (2000a); Aspects of the metabolism of dairy cows during the incubation of bovine spongiform encephalopathy. Vet Rec., 147(15); 409-412.
https://www.ncbi.nlm.nih.gov/pubmed/11072985
SMITH, B. P. (1996): Large Animal Internal Medicine, 2nd edition, 2040 pp.
THE BSE Inquiry (2000): Key conclusions, the cause of BSE. The BSE Inquiry, Vol 1, London: Stationery Office,
https://webarchive.nationalarchives.gov.uk/
ukgwa/20060802142310/http://www.bseinquiry. gov.uk/
https://www.encyclopedia.com/science/medical-magazines/bse-inquiry-report-executive-summary-key-conclusions
WILESMITH, J. W. – WELLS, G. A. H. – CRANWELL, M. P. – RYAN, J. B. M. (1988): Bovine spongiform encephalopathy; epidemiological studies. Vet Rec., 123(25), pp. 638–644, https://pubmed.ncbi.nlm.nih.gov/3218047/
Wilesmith,J.W.- Ryan,J.B.- Atkinson, M.J. (1991); Bovine spongiform encephalopathy: epidemiological studies on the origin. *Veter Rec*., 128 (9), pp. 199- 203 https://pubmed.ncbi.nlm.nih.gov/1823120/
WILESMITH, J. W. – RYAN, J. B. M. – HUESTON, W. D. (1992): Bovine spongiform encephalopathy; casecontrol studies of calf feeding practices and meatand- bone meal inclusion in proprietary concentrates. Res. Vet. Sci., 52(3), pp. 325–331, https://pubmed.ncbi.nlm.nih.gov/1620965/
WILESMITH, J. W. (2000): Statement No 91 Dr John Wilesmith (scheduled to give oral evidence 22/06/98). In: The BSE Inquiry, London: Stationery Office.