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President's Column

A recent report by the "Pew Research Center" describes how young people view their lives, futures and politics.

According to the report, a new generation has been shaped by an extraordinary revolution in technology and spectacular events both at home and abroad. They are "Generation Next", the group of young adults who have grown up with personal computers, cell phones and the internet and are now taking their place in a world where the only constant is rapid change.

So what are some of the traits of "Generation Next":

- Text messaging, instant messaging and email keep them in constant contact with work, family and friends.
- They are the "Look at Me" generation. They use Social networking sites like Facebook, MySpace and MyYearbook.
- About half of Gen Nexter's say the growing number of immigrants to the U.S. strengthens the country more than any generation.
- "Generation Next" is less critical of the government.
- They maintain close contact with parents and family.
- Their heroes are close and familiar.
- They are more comfortable with globalization and new ways of doing work and finally,
- Most Gen Nexter's say their generation's top goals are fortune and fame.

A full report can be downloaded at: www.people-press.org/reports/

Sergio F. Nates, Ph.D.

"If history and science have taught us anything, it is that passion and desire are not the same as truth."

—E.O. Wilson

Country focus - Chile (Sergio Nates)

Chile is one of the few countries in the world that are free from all major animal diseases that limit trade in animal products. Chile's animal health status is unique in the hemisphere and quite exceptional internationally. Chile is free from all major animal diseases, including Bovine Spongiform Encephalopathy (BSE) and it is also free from avian influenza.

Beef

In 2006, beef exports rose to $35 million dollars. The main destination markets are Mexico, Japan, Germany, and the United Kingdom, all countries with high quality standards.

Lamb

The largest lamb meat producing farms are located in the southernmost area of Chile, near the Strait of Magellan and *Tierra del Fuego* Island (Chilean Patagonia). This area is characterized by vast and unpolluted natural prairies that offer clean water, free from agrochemicals. Chile also produces a broad range of products such as small lamb carcasses, offals, bone-in or boneless fresh or frozen lamb meat cuts, vacuum packed or packed with film, in layers or blocks and in cardboard boxes of different sizes.

Pork

Pork production has experienced an outstanding growth over the last decade, with an increase of around 9.3 % annually. In 2006, 4,741,527 pigs were slaughtered, producing 467,866 tons of pork meat on the carcass. Today, 59% of the production of pork meat is destined for national consumption; however, the industry forecasts, in the next few years, to balance this participation by exporting. Today, Chile has recorded a sustained growth in pork meat exports, with average increases of 38% annually over the last four years, reaching highly demanding markets such as Japan, South Korea, Mexico and the European Union, among others. The industry aims to reach US$654 million in exports by 2010.

The Ministry of Agriculture, through the Agricultural and Livestock Service (SAG), is the Sanitary Authority in charge of inspecting and certifying the compliance of the integral system of quality control, which is based on enforcement procedures and regulations. Export Requirements to Chile can be found at:
http://www.fsis.usda.gov/regulations/Chile_Requirements/index.asp

R&D Update (Progress report)

06B-2 ***Production of Omega-3 Fatty Acid-Rich Algae from Animal Protein Hydrolysate(by Zhiyou Wen)***

Background

Animal proteins are major products of the rendering industry. The traditional market for these products, animal feed, is mature and in some cases threatening to shrink. For example, last year Canadian officials restricted specified risk material (SRM) and protein derived from SRM from from use in animal feeds, pet foods, and fertilizers; current EU regulations are even stricter, banning all ruminant tissue from feed for animals other than pets. Given these conditions and the possibility that the United States will further tighten feed regulations, there is an urgent need to

develop new markets for large quantities of rendered animal proteins.

The goal of this project is to develop an alternative for utilization of rendered animal protein by growing microalgae on animal protein hydrolysates to produce omega-3 polyunsaturated fatty acids, which have shown beneficial effects in preventing cardiovascular diseases, cancers, schizophrenia, and Alzheimer's. The traditional mass algal culture is limited by high cost of nitrogen sources such as peptone and tryptone. In order to develop a cost-effective mass algal culture process, a less expensive nitrogen source is desired, and animal protein hydrolysate provides such an opportunity.

Objectives

(1) Production of a range of hydrolysates which vary in terms of hydrolysis method, degree of hydrolysis, and raw material source;
(2) Analysis of peptides in terms of properties hypothesized to affect algal performance;
(3) Investigation of algal growth and omega-3 fatty acid production by using protein hydrolysate as a source of nitrogen and micronutrients;
(4) Characterization of the algal biomass produced.

Work conducted before this reporting period (January, 2007 – September, 2007)

We have reported the work that was done before this report period in previous progress report. In summary, it includes (1) preparation of alkali-hydrolysates of various animal protein sources, (2) determination of molecule size of these alkali-hydrolysates, (3) establishment of culture protocol of several algal/fungal species which can potential produce high level of omega-3 fatty acid from animal protein hydrolysates. The details of these accomplishments can be found from previous reports. The works conducted in this project period are continuations of those previous works.

Work conducted in this reporting period (October 2007 – March 2008)

1. Preparation of enzyme-hydrolysates of animal proteins

The enzymatic hydrolysates of animal proteins were prepared during this project period. We used three raw protein sources: meal and bone meal, blood meal, and feather meal. For each raw protein source, three different enzymes were used for hydrolysis: Alcalase, Flavourzyme, and Versazyme. Table 1 list the sample IDs and their corresponding preparation methods. The alkali-hydrolysates prepared previously was also presented in Table 1.

2. Characterization of both alkali- and enzyme-hydrolysates of animal proteins

Both the alkali-hydrolysates and enzyme-hydrolysates were characterized during this project period. The moisture contents of the samples were less than 5%. Alkali-hydrolysates have higher ash contents than the enzyme-hydrolysates. Hydrolysates from meat and bone meal, blood meal, and feather meal had a similar ash content and crude protein content, without significantly differences. Table 2 lists all the data for the above mentioned parameters.

3. Feasibility of producing omega-3 fatty acids from animal protein hydrolysates by algal/fungal fermentation.

A major effort conducted in this project period is to study the feasibility of using animal protein hydrolysates for growing algae or fungi which can produce high level of omega-3 fatty acids. In previous report, we reported that two species was identified as omega-3 producers: *Schizochytrium limacinum* as a DHA producer; *Phaeodactylum tricornutum* as an EPA producer. However, we later found that the

species *Phaeodactylum tricornutum* is not an ideal species because it required high level of light irradiation and accumulate relatively low level of EPA inside the cells when high content of animal protein hydrolysates was used. Therefore, *P. tricornutum* was eliminated from our candidate list. Instead, we selected a fungal species, *Pythium irregulare*, which has been reported to produce high level of EPA when growing on yeast extract. Our preliminary results have shown that this fungus can grow in medium containing animal protein hydrolysates, and produce relative high level of EPA. Therefore, we eventually chose *P. irregulare* as the EPA producer in this work.

During the project period, both the alkali- and enzyme-hydrolysates were tested for supporting the growth and omega-3 fatty acids production of *Schizochytrium limacinum* and *Pythium irregulare*. The results are reported as follows.

(i) Alkali-hydrolysates as a nitrogen source for supporting DHA-producer *S. limacinum*. We grew the alga *S. limacinum* in the medium containing alkali-hydrolysates as a nitrogen source. However, the growth performance was very poor compared with the yeast extract/peptone as nitrogen sources. We conclude that alkali-hydrolysates of animal proteins are not a suitable nitrogen source for growing the alga *S. limacinum* to produce DHA.

(ii) Alkali-hydrolysates as a nitrogen source for supporting EPA-producer *P. irregulare*. The fungus *P. irregulare* was also grown in medium containing alkali hydrolysates as a nitrogen source. The results are presented in Figure 1. Compared with the alga *S. limacinum*, the growth performance of *P. irregulare* was much better. Among three meals, feather meal hydrolysates supported the best cell growth. However, the EPA contents from all three meals were similar. Combining the cell biomass and EPA cellular content effects, we found that the high EPA production was obtained from feather meal. Based on the above results, we conclude that alkali hydrolysates of animal proteins can serve as a nitrogen sources for growing the fungus *P. irregulare* to produce EPA. Although the final EPA production level is still lower than the yeast extract culture (Figure 1), there are still plenty room for us to further increase the production level through process optimization.

(iii) Enzyme-hydrolysates as a nitrogen source for supporting DHA-producer *S. limacinum*. Currently, we are testing using the enzyme-hydrolysates for growing the alga *S. limacinum*. This is an ongoing work, and we will report the results in the next progress report.

(iv) Enzyme-hydrolysates as a nitrogen source for supporting EPA-producer *P. irregulare*. The enzyme hydrolysates were also used as a nitrogen source for the fungus *P. irregulare* to produce EPA. The results are shown in Figure 2. The fungus grew very well on meat and bone meal and feather meal, but the growth was poor on blood meal. Although the EPA cellular content from blood meal was little higher, the final EPA concentration in the culture solution was still low in the blood meal culture, due to the low cell biomass (Figure 2). The final EPA production from different meat and bone meal, and feather meal had a similar trend. It was also found that the EPA production levels from the meat and bone meal and feather meal were still somewhat lower than the yeast extract culture, however, this difference was not as significant as those in the alkali-hydrolysates culture (Figures 1 and 2). With the further process optimization, the production level from these two enzyme-hydrolysates can be increased.

Summary

In this project period, we produced enzyme-hydrolysate samples, and characterized both the alkali- and enzyme-hydrolysates. Then, we used the two types of hydrolysates to test their capabilities of supporting DHA production by *S. limacinum* and EPA production by *P. irregulare*. The results show that it is feasible to use both the alkali- and enzyme-hydrolysates for fungal culture to produce EPA. In terms of feasibility of using protein hydrolysates for supporting algal culture producing DHA, the alkali-hydrolysate cannot support the algal growth; the enzyme-hydrolysate is currently being tested. In the remaining project period, we will (i) optimize the process of using alkali- and enzyme-hydrolysates for fungal growth; (ii) finish the

feasibility study of using enzyme hydrolysate for algal growth, and (iii) further characterize the remaining protein hydrolysates sample and the resultant fungal/algal biomass that contain high level of omega-3 fatty acids.

Table 1. Sample ID codes for the alkali and enzymatic hydrolysates

	ID code	description
Alkali hydrolysates	MA4	meat and bone meal, alkaline hydrolysis, 4 hours
	MA8	meat and bone meal, alkaline hydrolysis, 8 hours
	MA16	meat and bone meal, alkaline hydrolysis,16 hours
	FA4	feather meal, alkaline hydrolysis, 4 hours
	FA8	feather meal, alkaline hydrolysis, 8 hours
	FA16	feather meal, alkaline hydrolysis,16 hours
	BA4	blood meal, alkaline hydrolysis, 4 hours
	BA8	blood meal, alkaline hydrolysis, 8 hours
	BA16	blood meal, alkaline hydrolysis,16 hours
Enzyme hydrolysates	BV	blood meal, versazyme hydrolysis,
	MV	meat and bone meal, versazyme hydrolysis,
	FV	feather meal, versazyme hydrolysis,
	B ALC	blood meal, alcalase hydrolysis,
	M ALC	meat and bone meal, alcalase hydrolysis,
	F ALC	feather meal, alcalase hydrolysis,
	B ALC FLA	blood meal, alcalase and flavourzyme hydrolysis,
	M ALC FLA	meat and bone meal, alcalase and flavourzyme hydrolysis,
	F ALC FLA	feather meal, alcalase and flavourzyme hydrolysis,

Table 2. Proximate analysis of animal protein hydrolysates (see **Table 1** for sample ID description)

Sample ID	Moisture content (%)	Ash content (%, dry basis)	Crude protein content (%,dry basis)
Alkali hydrolysates			
MA4	4.12 ± 0.18	19.65 ± 1.30	81.80 ± 1.03
MA8	2.95 ± 0.07	20.85 ± 1.41	80.50 ± 1.42
MA16	2.90 ± 0.17	22.85 ± 0.53	75.90 ± 2.18
FA4	2.30 ± 0.33	17.70 ± 0.18	87.90 ± 3.31
FA8	3.40 ± 0.62	20.82 ± 0.24	81.75 ± 1.36
FA16	2.51 ± 0.15	20.46 ± 0.12	85.04 ± 0.60
BA4	1.87 ± 0.25	27.16 ± 0.11	79.11 ± 1.94
BA8	2.99 ± 0.12	18.89 ± 0.01	79.63 ± 0.38
BA16	2.15 ± 0.33	14.75 ±0.20	69.33 ±0.89
Enzyme hydrolysates			
BV	2.32 ±0.07	8.46 ± 0.05	95.00 ± 0.70
MV	2.32 ±0.12	14.89 ± 0.48	77.74 ± 0.28
FV	1.36 ±0.11	11.05 ± 0.03	88.38 ± 2.27
B ALC	1.92 ±0.74	12.06 ± 0.33	84.60 ± 0.83
M ALC	1.96 ±0.07	14.19 ± 0.27	70.53 ± 0.53
F ALC	3.21 ±0.11	12.30 ± 0.11	79.33 ± 0.65
B ALC FLA	1.74 ±0.38	12.14 ± 0.20	76.44 ± 0.37
M ALC FLA	0.33 ±0.06	15.12 ± 0.13	66.02 ± 0.87
F ALC FLA	0.72 ±0.34	12.22 ± 0.08	70.18 ±0.91

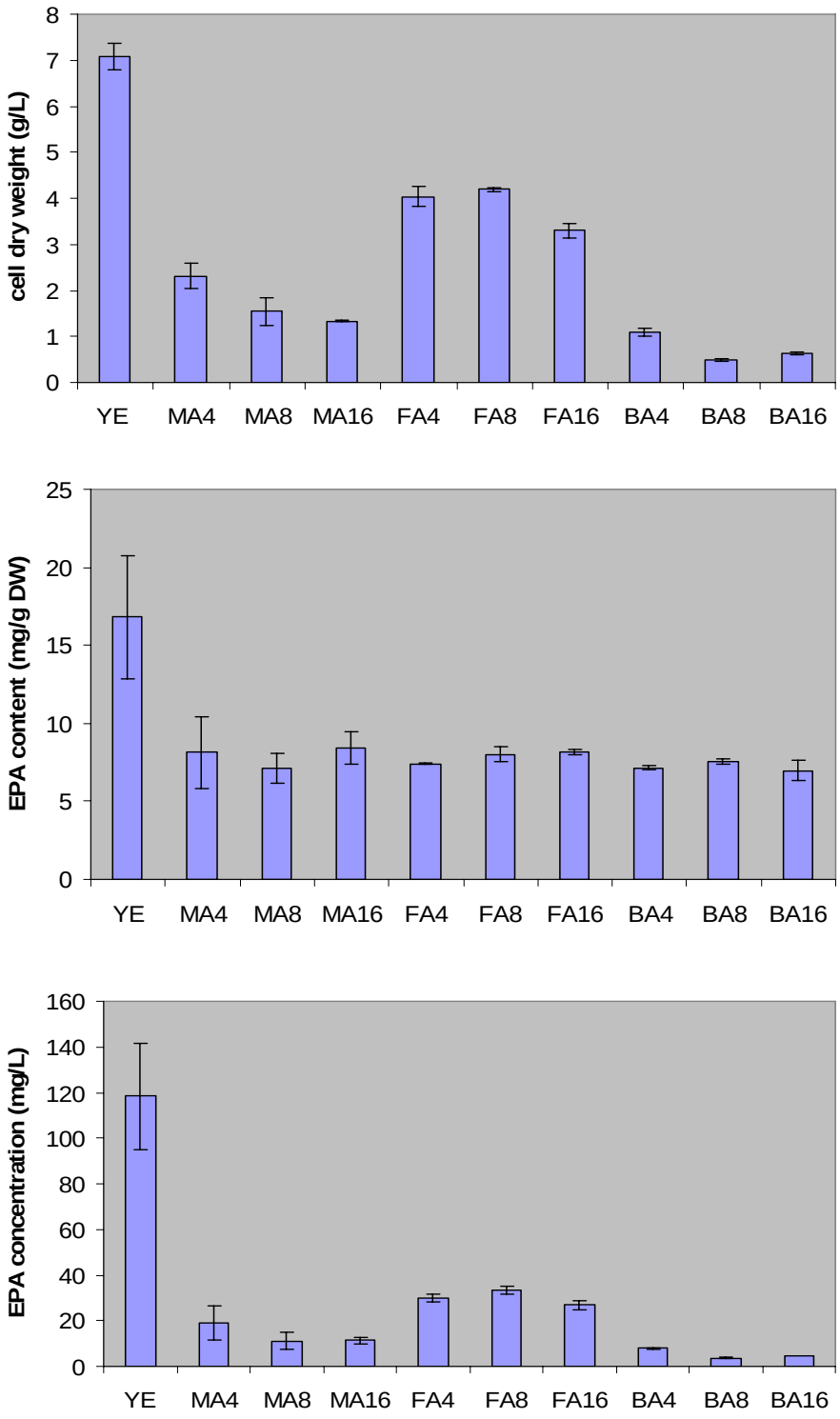


Figure 1. Cell growth, EPA content, and EPA concentration in the fungal culture with alkali-hydrolysates being used nitrogen sources. (see **Table 1** for sample ID description)

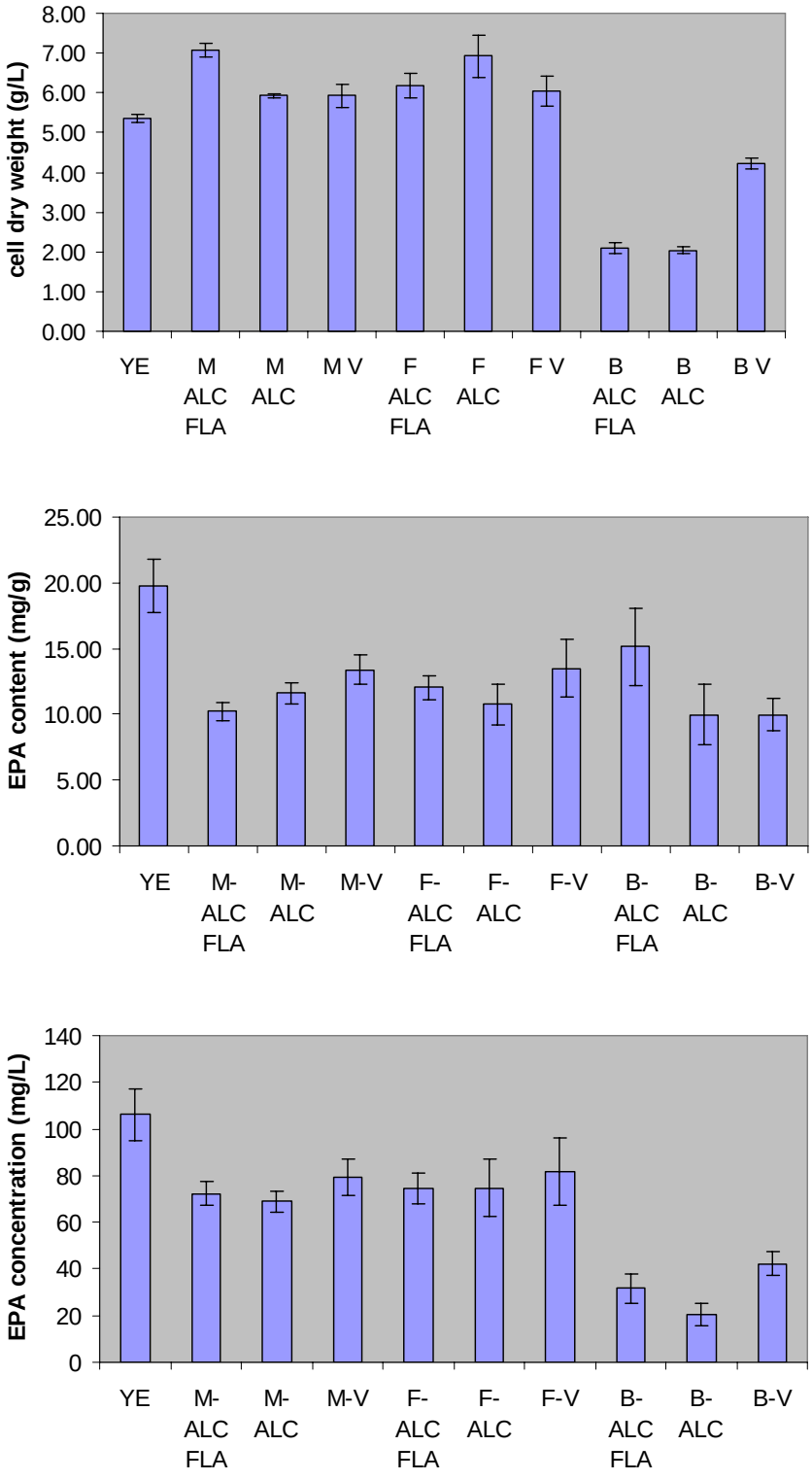


Figure 2. Cell growth, EPA content, and EPA concentration in the fungal culture with enzyme-hydrolysates being used nitrogen sources. (see **Table 1** for sample ID description)

Clemson Update

Since we have a column in the “Render Magazine” dedicated to keep the members informed about research projects at ACREC, the objective of this section is to update the members on other research centers and their research programs at Clemson University.

Among centers, the “Clemson University International Center for Automotive Research” (CU-ICAR) vision is to become the premier automotive and motor-sports research and education facility in the world.

The CU-ICAR campus is a 250 plus acre research campus and some of their partners include companies such as BMW, IBM, Michelin, Sun Mycrosystems, Microsoft, Bellsouth and Microsoft. The center is a public-private initiative with commitments of more than $215 million.

Additional information about the center can be found at:

www.clemson.edu/autoresearch/

Noteworthy Article

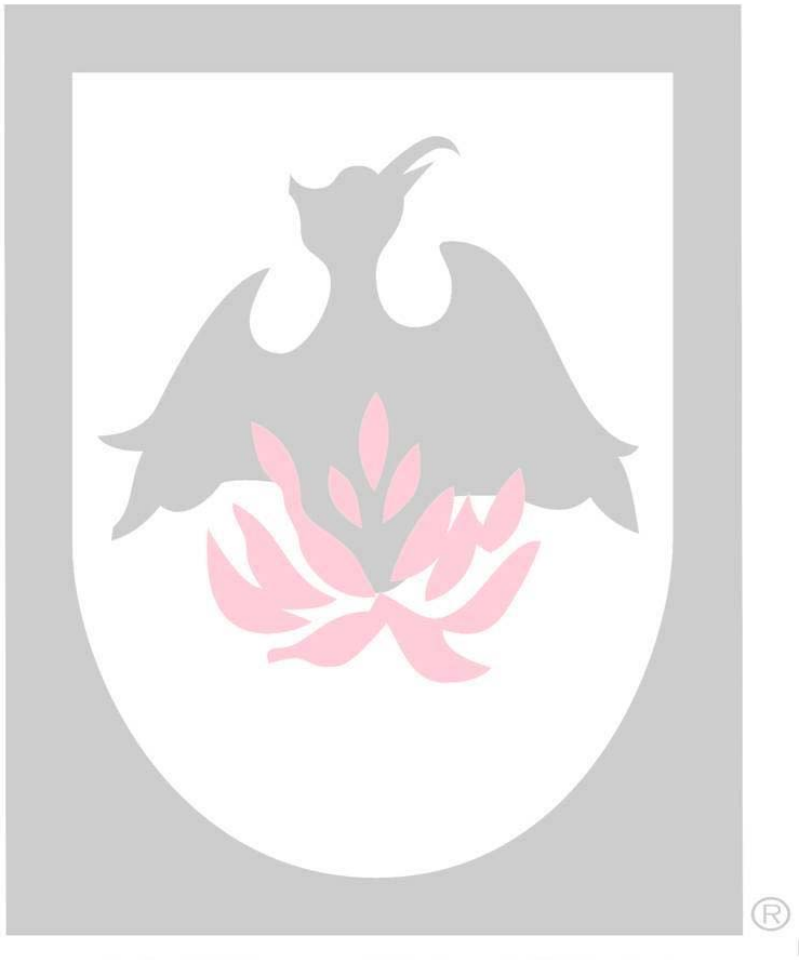
Ducrot, C., Arnold M., de Koeijer A., Heim D., and Clavas D. (2008). Review on the epidemiology and dynamics of BSE epidemics. Veterinary Research 39(4):15

The paper describes how the comprehensive surveillance of bovine spongiform encephalopathy (BSE) and studies carried out on these data has enhanced our knowledge of the epidemiology of BSE. Around 7 000 BSE cases were detected through the screening of about 50 million cattle with rapid tests in Europe. It confirmed that the clinical surveillance had a poor capacity to detect cases, and also showed the discrepancy of this passive surveillance efficiency between regions and production types (dairy/beef). Other risk factors for BSE were being in a dairy herd (three times more than beef), having a young age at first calving (for dairy cattle), being autumn-born (dairy and beef), and being in a herd with a very high milk yield.

These findings focus the risk on the feeding regimen of calves/heifers. Several epidemiological studies across countries suggest that the feed borne source related to meat and bone meal (MBM) is the only substantiated route of infection - even after the feed ban -, while it is not possible to exclude maternal transmission or milk replacers as a source of some infections. In most European countries, the average age of the cases is increasing over time and the prevalence decreasing, which reflects the effectiveness of control measures. Consistent results on the trend of the epidemic were obtained using back-calculation modeling, the R(0) approach and Age-Period-Cohort models. Furthermore, active surveillance also resulted in the finding of atypical cases.

These are distinct from previously found BSE and classified in two different forms based on biochemical characteristics; their prevalence is very low (36 cases up to

1st September 2007), affected animals were old and some of them displayed clinical signs. The origin and possibility of natural transmission is unknown.



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