ORIGINAL ARTICLE

Microbiological, physico-chemical and safety parameters of cereal-based animal diets

S. Paramithiotis[1], A.M. Pappa[2], E.H. Drosinos[1] & P.E. Zoiopoulos[2]

1 Laboratory of Food Quality Control and Hygiene, Department of Food Science and Technology, Agricultural University of Athens, Athens, Greece
2 Laboratory of Animal Production, School of Management of Natural Resources and Enterprises, University of Ioannina, Agrinio, Greece

Key words
feed manufacturing; HACCP; *Listeria*; microbiological quality; *Salmonella*; traceability.

Correspondence
Eleftherios H. Drosinos, Laboratory of Food Quality Control and Hygiene, Department of Food Science and Technology, Agricultural University of Athens, 75, Iera Odos, Street, Votanicos, GR-118 55 Athens, Greece
Tel: +30 210 529 4713
FAX: +30 210 529 4683
Email: ehd@aua.gr

Received 14 May 2009;
Accepted 15 July 2009

doi:10.1111/j.1757-837X.2009.00028.x

Abstract

Introduction Feed hygiene is important for the safety of foods of animal origin. Feed hazards include mycotoxins and pathogenic bacteria responsible for food-borne diseases. The aim of this study was to evaluate the safety of samples consisting of compound feeds, feed materials and premixes, and provide information for the food chain. *Methods* Three series of samples were taken for analyses at monthy intervals from 25 batches of various types of feeds (75 in total) at a feed manufacturers. Standard and established methods were used for both microbiological and physico-chemical analyses. *Results* The water activity of the samples ranged from 0.578 to 0.648 and 0.659 to 0.741, whereas pH ranged from 5.78 to 6.19 and 5.82 to 6.41 in loose and pelleted compound feeds, respectively. The total bacterial count in loose feeds ranged from 4.44 to 6.30, yeasts–moulds 3.30 to 4.07, *Enterobacteriaceae* 3.23 to 4.74 and coliforms 3.21 to 4.89 $\log \mathrm{CFU\,g^{-1}}$. Total bacterial count in pelleted feeds ranged from < 2.0 to 3.7 $\log \mathrm{CFU\,g^{-1}}$, whereas values for other variables were negligible. Wheat bran was most heavily loaded with microbes. *Staphylococcus aureus* and aflatoxin were not found in any of the 75 samples, whereas *Escherichia coli* was detected in soybean, sunflower and three out of 30 samples of compound feeds. *Listeria* spp. was found in only one out of three batches of sugarbeet pulp and in one out of three batches of two pelleted feed not containing sugarbeet pulp. *Salmonella* spp. was detected in two out of 15 samples of loose feeds. *Conclusion* These data meet demands of recent European Union legislation on feed hygiene for establishing specific microbiological criteria for feed manufacturers and fill gaps on the traceability and development of the Hazard Analysis Critical Control Points system in the animal production sector.

Introduction

Farm animals and feedstuffs are the basis of the production of food of animal origin. Feedstuffs are not only a source of energy and nutrients (Coleman & Moore, 2003) but can also influence the quality of food in a variety of ways, through the presence of undesirable substances that they may contain. Therefore, particular attention must be paid to the absolute safety of feedstuffs for animals and the consumer (Petersen & Flachowsky, 2004; Flachowsky & Danicke, 2005). Following the food crises (BSE scandal, dioxine episode, opinion dichotomy over GMOs, antibiotic cross-resistance, etc.) in the second half of 1990s, the European Union (EU) adopted a fundamental piece of legislation, namely the 'General Food Law' (EU, 2002a), which raised animal feed up to the same level as that of food for humans. This Regulation, among others, introduced the element of traceability in the food chain. Furthermore, recently, EU adopted a very important Regulation on feed hygiene (EU, 2005). Feed hygiene plays a significant role in the safety of foods of animal origin (Kan & Meijer, 2007). Feed hazards include, among others, the presence of mycotoxins and the growth of pathogenic bacteria such as *Salmonella* and *Listeria,* responsible for food-borne diseases (Sofos, 2006). Although the issue of food microbiological safety has been extensively studied, however, there is a lack of information

on feed microbiological safety (Smulders *et al.*, 2006). The Community law for Feed Hygiene states that feed manufactures plan, apply and maintain permanent written procedures based on the principles of Hazard Analysis Critical Control Points (HACCP).

The present study constitutes an investigation to obtain data, related to food hygiene, based on physico-chemical and microbiological parameters of feed material, premixes and compound feeds, in different stages of feed flow at a feed manufacturing establishment. These data will serve as an essential source of information to the traceability concept, developing GMP and also as an important prerequisite to the establishment of HACCP systems for the animal production sector in Greece and elsewhere.

Materials and methods

Sampling

Samples were obtained from a feed manufacturer's establishment located in Greek mainland. A total of 75 samples, were taken from three different stages of the compound feed production process, i.e. storing of feed materials and premixes, mixing and storing of the final compound feeds (Figure 1). Thirty-three feed materials, 12 premixes, 15 loose compound feeds and 15 samples of the corresponding final pelleted compound feeds were analysed. The individual feed materials, premixes, as well as the composition of the loose and pelleted compound feeds studied are shown in Table 1. Samples were collected from three different batches at monthly intervals during feed processing in order to obtain more representative data. The results of each batch are given separately for traceability reasons. Collection of samples was performed according to established Community methods (EEC, 1976). The collected samples were sent to the laboratory for analyses on the same day.

Physico-chemical analyses

The pH value of the samples was determined according to ADAS (1986) by suspending 50 g of the sample in 125 mL distilled water. The mixture was kept under constant agitation for 1 h at room temperature and then pH was measured by directly immersing the electrode of a pH meter (Knick Elektronische Messgerate, Berlin, Germany). The water activity value of the samples was determined using a Rotronic Hygrolab (Rotronic, Instrument Corp., New York, NY, USA) according to the manufacturer's instructions. Aflatoxin B_1 determination was performed using a Veratox® HS quantitative aflatoxin B_1 high-sensitivity test (Neogen Corporation, Lansing, MI, USA) according to the manufacturer's instructions, with a limit of detection of 1.0 p.p.b. In case of the presence of aflatoxin B_1, samples were subjected to HPLC analysis according to ISO 14718 (1998).

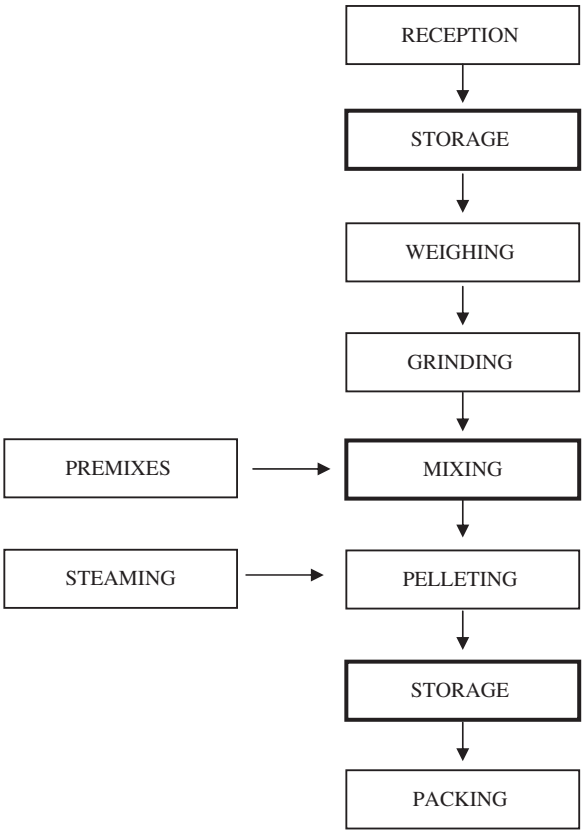

Figure 1 Flow diagram of the feed manufacturing establishment. Sampling sites, i.e. storage of ingredients, mixing and storage of the final product are indicated by bold frames.

Microbiological analyses

The sample (25 g) was aseptically homogenized with 225 mL of sterile saline containing 0.1% (w/v) peptone (Merck, Darmstadt, Germany) and 0.85% (w/v) NaCl (Merck) using a stomacher apparatus (Seward Medical, London, UK). Serial dilutions were performed in sterile Ringer solution (Merck). The pour plating technique was performed by mixing 1 mL of the appropriately diluted sample with molten media. The surface spreading technique was performed by spreading 0.1 mL of the appropriately diluted sample to the surface of the media. In all cases, duplicate plates were prepared. The total bacterial count (TBC) (aerobic mesophilic) was estimated by spreading on plate count agar (Merck) and incubating at 30 °C for 48 h. Total coliforms and *Escherichia coli* were determined and distinguished by pouring in chromocult® agar (Merck),

Table 1 Composition of compound feeds (g/kg)

Feeds	Pig grower	Pig fattener	Sow lactation	Calf fattener	Ewe lactation
Feed materials					
Maize grain	250	175	275	280	308
Barley grain	50	117	75	189	–
Wheat grain	317	366	237	–	–
Soyabean meal	247	215	271	20	218
Sunflower meal	–	–	–	168	125
Wheat bran	80	80	70	175	141
Dried Citrus Pulp	–	–	–	50	50
Dried sugarbeet pulp	–	–	–	75	100
Soya oil	15	7	25	–	–
Fat	–	–	–	–	12
Limestone	8	7	14	10	13
Premixes					
Pig grower and fattener	33	33	–	–	–
Sow lactation	–	–	33	–	–
Ewe lactation	–	–	–	–	33
Calf fattener	–	–	–	33	–

according to the manufacturer's instructions, and incubated at 35 °C for 24 h. Yeasts and moulds were determined by surface spreading on yeast glucose chloramphenicol agar (Merck) and incubation at 25 °C for 48 h. *Enterobacteriaceae* determination was performed by pouring in violet red bile glucose agar (Biolife, Milano, Italy) and incubation at 37 °C for 48 h. *Staphylococcus aureus* determination was carried out by spreading on Baird–Parker selective agar (Merck) and incubation at 35 °C for 24–48 h. Qualitative determination of *Listeria* sp. and *Salmonella* sp. was performed as follows: in the former case, a pre-enrichment in Fraser broth (Merck) was performed and then inoculation on Palcam agar (Biolife) at 35 °C for 48 h, whereas in the latter case, the pre-enrichment step in buffered peptone water was followed by enrichment in RVS broth (Merck) and then inoculation on XLD agar (Merck) at 35 °C for 48 h according to the manufacturer's instructions.

Results and discussion

Physico-chemical analyses

The water activity and pH values of the feed materials, premixes as well as compound feeds, in loose and pelleted forms are shown in Table 2. Both water activity and pH values exhibited a considerable variation among different batches within the same feed material. The lowest a_w value recorded was 0.355, corresponding to the third batch of dried citrus pulp, although the respective value from the first batch of the same feed material was 0.450. In addition, the water activity values of different batches of limestone ranged from 0.600 to 0.454. Similar differences were also observed for the majority of feed materials. The highest a_w value recorded was 0.686, corresponding to the third batch of wheat bran. Overall, the pH values ranged from 3.67 to 9.76, with ewe lactation premix and limestone exhibiting the lowest and the highest values, respectively. pH fluctuation in general was low, with the exception of fat and premixes for pigs and ewes. The variation of pH and a_w of loose and pelleted compounds feeds was also low. pH and a_w values of the loose compound feeds ranged from 5.78 to 6.19 and from 0.578 to 0.648, respectively. On the other hand, a_w values of the pelleted compound feeds were higher than the respective loose ones, ranging from 0.659 to 0.741, while pH values ranged from 5.82 to 6.41.

The variation observed in both a_w and pH values of the feed materials used can be explained by the lack of standardized conditions in the primary production of raw materials. It should be stressed that maintenance of these low a_w values requires proper post-harvesting handling of raw materials, so that absorption of moisture by the feeds can be avoided. Given the variation in a_w that has been observed with dried citrus pulp, particular care should be taken with this ingredient, because of its content in hydrophilic pectin (Gohl, 1981). On the other hand, a_w and pH values of the loose and pelleted compounds feeds exhibited considerably lower variation, both between different batches but also between the various feed types. This was due to the homogeneous mixing in the former, combined with the beneficial effect of pelleting in the latter case. The higher a_w values of the final pelleted products compared with the respective loose ones are due to the steam added for pellet formation.

Microbiological analyses

Data of the microbiological quality of feed materials, premixes and compound feeds in loose and pelleted form are shown in Tables 3–5, respectively. The highest microbial load, in terms of TBC, yeast–mould count, *Enterobacteriaceae* and coliforms count, was observed in all batches of wheat bran. In contrary, the lowest microbial load was observed in limestone, soya oil and fat, where counts were below the detection limit. *E. coli* was detected in all batches of soybean meal and in the first batch of sunflower meal. No *S. aureus* or *Salmonella* sp. presence was observed in either feed materials or premixes. On the other hand, the presence of *Listeria* sp. has been detected in the first batch of dried

Table 2 Water activity (a_w) and pH values of feed materials, premixes and compound feeds in loose and pelleted form.

	a_w			pH		
Feeds	Batch 1	Batch 2	Batch 3	Batch 1	Batch 2	Batch 3
Feed materials						
Maize grain	0.619 (0.004)	0.613 (0.003)	0.666 (0.004)	6.03 (0.04)	5.85 (0.02)	5.87 (0.04)
Barley grain	0.656 (0.003)	0.655 (0.002)	0.656 (0.002)	6.07 (0.02)	6.05 (0.04)	6.07 (0.03)
Wheat grain	0.618 (0.003)	0.525 (0.003)	0.588 (0.003)	6.25 (0.04)	6.32 (0.03)	6.29 (0.02)
Soyabean meal	0.654 (0.003)	0.648 (0.002)	0.645 (0.003)	6.44 (0.01)	6.66 (0.05)	6.65 (0.02)
Sunflower meal	0.556 (0.002)	0.548 (0.002)	0.501 (0.003)	6.26 (0.02)	6.27 (0.02)	6.48 (0.03)
Wheat bran	0.651 (0.002)	0.675 (0.001)	0.686 (0.002)	6.68 (0.04)	6.50 (0.02)	6.66 (0.03)
Dried citrus pulp	0.450 (0.001)	0.449 (0.002)	0.355 (0.002)	8.40 (0.03)	8.37 (0.03)	8.35 (0.04)
Dried sugarbeet pulp	0.656 (0.001)	0.589 (0.002)	0.588 (0.003)	6.09 (0.02)	5.92 (0.04)	6.05 (0.03)
Soya oil	0.544 (0.001)	0.531 (0.003)	0.523 (0.002)	6.60 (0.02)	6.58 (0.03)	6.50 (0.11)
Fat	0.584 (0.002)	0.620 (0.003)	0.674 (0.003)	5.37 (0.08)	5.24 (0.07)	4.45 (0.09)
Limestone	0.600 (0.002)	0.585 (0.002)	0.454 (0.003)	9.76 (0.05)	9.74 (0.03)	9.45 (0.04)
Premixes						
Pig grower and fattener	0.545 (0.002)	0.523 (0.001)	0.546 (0.002)	4.29 (0.02)	4.83 (0.03)	4.73 (0.02)
Sow lactation	0.512 (0.002)	0.556 (0.003)	0.585 (0.002)	4.15 (0.03)	3.99 (0.04)	4.29 (0.05)
Ewe lactation	0.596 (0.002)	0.556 (0.002)	0.592 (0.003)	3.99 (0.04)	3.67 (0.03)	4.44 (0.02)
Calf fattener	0.534 (0.001)	0.532 (0.002)	0.530 (0.003)	4.19 (0.02)	4.20 (0.03)	4.15 (0.04)
Loose compounds						
Pig grower	0.648 (0.002)	0.625 (0.002)	0.615 (0.002)	6.02 (0.04)	5.99 (0.03)	6.05 (0.03)
Pig fattener	0.630 (0.003)	0.615 (0.003)	0.629 (0.002)	6.10 (0.03)	6.07 (0.03)	6.19 (0.02)
Sow lactation	0.619 (0.004)	0.628 (0.003)	0.618 (0.002)	5.98 (0.02)	6.06 (0.03)	5.93 (0.02)
Ewe lactation	0.609 (0.002)	0.578 (0.003)	0.603 (0.003)	5.78 (0.01)	5.88 (0.03)	6.05 (0.03)
Calf fattener	0.585 (0.003)	0.613 (0.004)	0.589 (0.002)	6.01 (0.05)	6.05 (0.01)	6.01 (0.03)
Pelleted compounds						
Pig grower	0.704 (0.003)	0.679 (0.002)	0.669 (0.002)	6.13 (0.02)	6.22 (0.02)	6.41 (0.02)
Pig fattener	0.697 (0.003)	0.674 (0.001)	0.688 (0.003)	6.11 (0.02)	6.20 (0.02)	6.36 (0.04)
Sow lactation	0.741 (0.001)	0.711 (0.003)	0.711 (0.002)	6.06 (0.03)	6.05 (0.02)	6.18 (0.03)
Ewe lactation	0.714 (0.002)	0.616 (0.003)	0.659 (0.002)	5.93 (0.03)	5.82 (0.04)	5.81 (0.03)
Calf fattener	0.702 (0.002)	0.673 (0.002)	0.674 (0.002)	5.87 (0.03)	5.83 (0.05)	5.86 (0.03)

All determinations were performed in triplicate. Standard deviation is given in parenthesis.

sugarbeet pulp. Both qualitative and quantitative differences of the microbial populations have been observed between batches. Sunflower meal, citrus pulp, sugar beet pulp and soybean meal exhibited certain qualitative differences. In this respect, *Enterobacteriaceae*, coliforms and *E. coli* were detected only in the first batch of sunflower meal, whereas yeast and mould counts were not found in two batches of citrus pulp, sugarbeet pulp and one of soybean meal. On the other hand, differences between batches have been observed for sunflower meal, sugarbeet pulp, wheat grain and sow lactation premix, mainly in terms of the TBC.

The variation of the microbiological quality of the feed materials and premixes that has been observed can mainly be attributed to their storage conditions. The inability to correlate the pH and a_w values of the raw materials and premixes with their microbial quality shows the importance of the composition of the feed matrix in supporting the microbial growth (Maciorowski *et al.*, 2007). The characterization of the microbial load and its changes in liquid piglet feed has been reported by Plumed-Ferrer *et al.* (2004) but due to the different nature of the feed (liquid versus concentrates in our study), it is difficult to extrapolate the results. However, the microbiological quality of barley, wheat and maize grains has also been assessed by Vlachou *et al.* (2004). Compared with their findings, in the case of barley and wheat grains a similar situation was noted regarding microbiological quality, whereas maize grains, analysed in our study, appeared to be of better microbiological quality, in terms of the total bacterial and yeast and mould counts. Furthermore, no *Salmonella* sp. has been detected in the present study, in contrast to the results obtained by Vlachou *et al.* (2004), where its presence had been verified in two out of 138 feed materials (1.4%) and none of 73 compound feeds, all of plant origin. McIlroy (2001), commenting on biosecurity programmes for *Salmonella* control, referred to data published by the Ministry of

Table 3 Microbiological parameters of feed materials

Feed materials	TBC	Yeasts–moulds	*Enterobacteriaceae*	Coliforms	*Staphylococcus aureus*	*Escherichia coli*	*Listeria* sp.	*Salmonella* sp.	Aflatoxin B_1
Maize grain									
Batch 1	2.26 (0.12)	2.31 (0.14)	2.41 (0.26)	< 1.00	< 2.00	< 1.00	Absence	Absence	< 1.0 p.p.b.
Batch 2	2.39 (0.28)	2.34 (0.20)	2.38 (0.26)	< 1.00	< 2.00	< 1.00	Absence	Absence	< 1.0 p.p.b.
Batch 3	2.41 (0.30)	2.43 (0.25)	2.25 (0.14)	< 1.00	< 2.00	< 1.00	Absence	Absence	< 1.0 p.p.b.
Barley grain									
Batch 1	5.17 (0.14)	3.44 (0.35)	4.14 (0.28)	4.34 (0.41)	< 2.00	< 1.00	Absence	Absence	< 1.0 p.p.b.
Batch 2	5.02 (0.31)	3.40 (0.29)	4.10 (0.36)	4.25 (0.32)	< 2.00	< 1.00	Absence	Absence	< 1.0 p.p.b.
Batch 3	5.10 (0.25)	3.47 (0.34)	4.20 (0.35)	4.36 (0.42)	< 2.00	< 1.00	Absence	Absence	< 1.0 p.p.b.
Wheat grain									
Batch 1	5.81 (0.14)	3.20 (0.10)	4.39 (0.26)	3.47 (0.24)	< 2.00	< 1.00	Absence	Absence	< 1.0 p.p.b.
Batch 2	4.66 (0.30)	3.41 (0.31)	4.43 (0.21)	3.10 (0.36)	< 2.00	< 1.00	Absence	Absence	< 1.0 p.p.b.
Batch 3	4.78 (0.38)	3.32 (0.25)	4.21 (0.47)	3.14 (0.25)	< 2.00	< 1.00	Absence	Absence	< 1.0 p.p.b.
Soyabean meal									
Batch 1	5.11 (0.37)	2.68 (0.24)	3.35 (0.14)	3.25 (0.14)	< 2.00	3.27 (0.18)	Absence	Absence	< 1.0 p.p.b.
Batch 2	4.52 (0.23)	< 2.00	3.30 (0.32)	3.14 (0.32)	< 2.00	2.97 (0.32)	Absence	Absence	< 1.0 p.p.b.
Batch 3	4.68 (0.36)	2.87 (0.38)	2.90 (0.16)	2.87 (0.16)	< 2.00	2.67 (0.28)	Absence	Absence	< 1.0 p.p.b.
Sunflower meal									
Batch 1	4.38 (0.38)	3.71 (0.42)	2.68 (0.15)	2.77 (0.47)	< 2.00	2.65 (0.32)	Absence	Absence	< 1.0 p.p.b.
Batch 2	2.62 (0.34)	2.20 (0.36)	< 1.00	< 1.00	< 2.00	< 1.00	Absence	Absence	< 1.0 p.p.b.
Batch 3	2.87 (0.36)	2.07 (0.23)	< 1.00	< 1.00	< 2.00	< 1.00	Absence	Absence	< 1.0 p.p.b.
Wheat bran									
Batch 1	5.39 (0.10)	3.34 (0.20)	4.36 (0.30)	4.53 (0.36)	< 2.00	< 1.00	Absence	Absence	< 1.0 p.p.b.
Batch 2	5.84 (0.21)	4.34 (0.25)	5.47 (0.42)	4.32 (0.34)	< 2.00	< 1.00	Absence	Absence	< 1.0 p.p.b.
Batch 3	5.35 (0.31)	3.92 (0.45)	5.03 (0.35)	4.61 (0.48)	< 2.00	< 1.00	Absence	Absence	< 1.0 p.p.b.
Citrus pulp									
Batch 1	2.84 (0.14)	< 2.00	< 1.00	< 1.00	< 2.00	< 1.00	Absence	Absence	< 1.0 p.p.b.
Batch 2	2.80 (0.20)	< 2.00	< 1.00	< 1.00	< 2.00	< 1.00	Absence	Absence	< 1.0 p.p.b.
Batch 3	2.63 (0.32)	2.35 (0.21)	< 1.00	< 1.00	< 2.00	< 1.00	Absence	Absence	< 1.0 p.p.b.
Sugarbeet pulp									
Batch 1	3.78 (0.38)	2.60 (0.42)	< 1.00	< 1.00	< 2.00	< 1.00	Presence	Absence	< 1.0 p.p.b.
Batch 2	2.25 (0.24)	< 2.00	< 1.00	< 1.00	< 2.00	< 1.00	Absence	Absence	< 1.0 p.p.b.
Batch 3	2.20 (0.21)	< 2.00	< 1.00	< 1.00	< 2.00	< 1.00	Absence	Absence	< 1.0 p.p.b.
Soya oil									
Batch 1	< 2.00	< 2.00	< 1.00	< 1.00	< 2.00	< 1.00	Absence	Absence	< 1.0 p.p.b.
Batch 2	< 2.00	< 2.00	< 1.00	< 1.00	< 2.00	< 1.00	Absence	Absence	< 1.0 p.p.b.
Batch 3	< 2.00	< 2.00	< 1.00	< 1.00	< 2.00	< 1.00	Absence	Absence	< 1.0 p.p.b.

Table 3 Continued

Feed materials	TBC	Yeasts–moulds	*Enterobacteriaceae*	Coliforms	*Staphylococcus aureus*	*Escherichia coli*	*Listeria* sp.	*Salmonella* sp.	Aflatoxin B_1
Fat									
Batch 1	< 2.00	< 2.00	< 1.00	< 1.00	< 2.00	< 1.00	Absence	Absence	< 1.0 p.p.b.
Batch 2	< 2.00	< 2.00	< 1.00	< 1.00	< 2.00	< 1.00	Absence	Absence	< 1.0 p.p.b.
Batch 3	< 2.00	< 2.00	< 1.00	< 1.00	< 2.00	< 1.00	Absence	Absence	< 1.0 p.p.b.
Limestone									
Batch 1	< 2.00	< 2.00	< 1.00	< 1.00	< 2.00	< 1.00	Absence	Absence	< 1.0 p.p.b.
Batch 2	< 2.00	< 2.00	< 1.00	< 1.00	< 2.00	< 1.00	Absence	Absence	< 1.0 p.p.b.
Batch 3	< 2.00	< 2.00	< 1.00	< 1.00	< 2.00	< 1.00	Absence	Absence	< 1.0 p.p.b.

Numbers of log CFU g^{-1}. All determinations were performed in triplicate. Standard deviation is given in parenthesis.
TBC, total bacterial count.

Agriculture, Fisheries and Foods in the United Kingdom, where between 5% and 10 % vegetable proteins were found to be contaminated with *Salmonella*. Another survey of cattle feeds in United States (Krytenburg *et al.*, 1998) reported *Salmonella* prevalence of 9.8% overall. In addition, a study of feed meals in the U.K. found *Salmonella* to be present in 8.4% of animal feed samples (Davies & Wray, 1997). Finally, Davis *et al.* (2003) reported that only 0.8% of feed materials and none of the compound feeds for cattle production tested were found positive for *Salmonella*.

The microbiological quality of loose and pelleted compound feeds is shown in Table 5. In the loose feeds, TBC, yeast–mould, *Enterobacteriaceae* and coliform populations were similar, ranging from 4.44 to 6.30, 3.30 to 4.07, 3.23 to 4.74 and 3.21 to 4.89 log CFU g^{-1}, respectively. *E. coli* was detected only in three cases: i.e. the first batches of loose compound feeds for pig fattening, calf fattening and lactating ewes. *S. aureus* and *Listeria* sp. presence was not detected. It is interesting to note that in two samples, namely the second batches of feed for growing pigs and calf fattening, the presence of *Salmonella* sp. has been recorded.

The microbial load of the pelleted compound feeds was considerably lower. In this respect, TBC ranged from 2.17 to 3.70 log CFU g^{-1}, but for calf fattening feed counts were not detectable. Members of the *Enterobacteriaceae* family were detected only in the middle batch of two feeds, namely pellet for growing and finishing pigs, and coliforms only in one batch of feed for growing pigs. Finally, it is interesting to record the presence of *Listeria* sp. in two cases, i.e. the middle batch of pellets for growing and finishing pigs.

The microbial load recorded for loose compound feeds can be explained by the microbial populations of the respective raw materials. The microbial populations of the main ingredients of Table 3, in particular wheat grain, soyabean meal, barley grain and wheat bran, apart from maize grain, which were relatively high, reflect the respective populations of the loose compound feeds.

Comparing microbial populations of the final pelleted products with the loose ones from the mixing equipment, a considerable decrease is observed in the former due to the beneficial effect of the hot (steam) pelleting that has taken place (McDonald *et al.*, 1995). This thermal treatment seemed to be capable of destroying *Salmonella* sp. cells because no *Salmonella* was detected in the final pelleted product. On the other hand, the presence of *Listeria* sp. in the final product can be explained by contamination from the environment. Sales and Yoshizawa (2006) confirm the presence of *Aspergillus flavus* in dusts generated by agricultural processing facilities including feed mill.

Table 4 Microbiological parameters of premixes

Premixes	TBC	Yeasts–moulds	*Enterobacteriaceae*	Coliforms	*Staphylococcus aureus*	*Escherichia coli*	*Listeria* sp.	*Salmonella* sp.	Aflatoxin B_1
Pig grower and fattener									
Batch 1	3.66 (0.25)	< 2.00	2.23 (0.15)	2.17 (0.10)	< 2.00	< 1.00	Absence	Absence	< 1.0 p.p.b.
Batch 2	2.90 (0.21)	< 2.00	2.32 (0.10)	2.23 (0.21)	< 2.00	< 1.00	Absence	Absence	< 1.0 p.p.b.
Batch 3	3.24 (0.28)	< 2.00	2.31 (0.14)	2.35 (0.23)	< 2.00	< 1.00	Absence	Absence	< 1.0 p.p.b.
Sow lactation									
Batch 1	5.14 (0.41)	2.34 (0.23)	2.23 (0.14)	< 1.00	< 2.00	< 1.00	Absence	Absence	< 1.0 p.p.b.
Batch 2	3.62 (0.29)	2.69 (0.17)	2.59 (0.30)	< 1.00	< 2.00	< 1.00	Absence	Absence	< 1.0 p.p.b.
Batch 3	4.53 (0.26)	2.47 (0.24)	2.35 (0.26)	< 1.00	< 2.00	< 1.00	Absence	Absence	< 1.0 p.p.b.
Ewe lactation									
Batch 1	4.04 (0.31)	< 2.00	< 1.00	< 1.00	< 2.00	< 1.00	Absence	Absence	< 1.0 p.p.b.
Batch 2	3.14 (0.16)	< 2.00	< 1.00	< 1.00	< 2.00	< 1.00	Absence	Absence	< 1.0 p.p.b.
Batch 3	3.64 (0.28)	< 2.00	< 1.00	< 1.00	< 2.00	< 1.00	Absence	Absence	< 1.0 p.p.b.
Calf fattener									
Batch 1	5.77 (0.36)	< 2.00	< 1.00	< 1.00	< 2.00	< 1.00	Absence	Absence	< 1.0 p.p.b.
Batch 2	5.56 (0.47)	< 2.00	< 1.00	< 1.00	< 2.00	< 1.00	Absence	Absence	< 1.0 p.p.b.
Batch 3	5.63 (0.42)	< 2.00	< 1.00	< 1.00	< 2.00	< 1.00	Absence	Absence	< 1.0 p.p.b.

Numbers of log CFU g^{-1}. All determinations were performed in triplicate. Standard deviation is given in parenthesis.
TBC, total bacterial count.

Aflatoxin analyses

Analyses for aflatoxin B_1 content revealed the total absence or, at least, the presence of this contaminant below the detection limit of the technique used (1.0 p.p.b.), in all samples of raw materials, premixes, and compound feeds both in loose and pelleted form. The presence of mycotoxins has been reported for various feed materials and in numerous places throughout the world (Fink-Gremmels, 2006). It is interesting to note that Vlachou *et al*. (2004), after having carried out a survey in Greece, reported that only seven out of 183 raw materials and none of 119 compound feeds were positive for aflatoxin B_1. In fact, in six out of the seven positive raw materials, the content of aflatoxin B_1 was only 10 p.p.b., a level that was lower than the EU maximum permitted limit of 20 p.p.b. (EU, 2002b). The same authors concluded that, in general, aflatoxin B_1 does not seem to constitute a problem for animal feeds in Greece, which was confirmed by the absence of this undesirable substance in 75 samples of feed materials, premixes and compound feeds in the present study.

Conclusions

The purpose of the present study was to characterize the microbial load that occurred in the various types of feed materials, premixes and compound feeds used at different stages of the production chain in a feed manufacturing factory. It should be emphasized that this investigation did not take place on an experimental farm, but on a commercial enterprise, and hence represents usual hygiene conditions. Despite the absence of aflatoxin B_1 from all feed samples, our results revealed the presence of *Listeria* spp. in one feed material and two pelleted diets, diets that did not contain this particular ingredient. Although the presence of *Listeria monocytogenes* has been reported in canned corn (Aureli *et al*., 2000) and silage (Nightingale *et al*., 2004), followed by clinical health problems of humans and ruminants who consumed them, respectively, to our knowledge, similar data for concentrated feed materials and compound feeds have not been reported in the literature. Very recently, Maciorowski *et al*. (2007) postulated that *Listeria* spp. may become a huge problem to the animal feed industry in the future.

In addition, *Salmonella* was detected in two batches of loose compound feeds. These findings can only be attributed to environmental contamination and this is particularly useful in establishing the HACCP system (Flachowsky & Danicke, 2005). Although the presence of *Salmonella* is associated with products of animal origin, it appears from our results that feed from plant origin constitutes a potential source of *Salmonella* infection and this issue should be investigated further. Animal feeding plays an essential role in *Salmonella* control, because it might be a potential carrier and infection source and also because effective measures can be applied at this stage to control bacterial transmission. The microbiological quality of feeds is a requirement in any *Salmonella* control programme (Coma, 2003).

Table 5 Microbial characteristics of loose and pelleted compound feeds

	TBC	Yeasts–moulds	*Enterobacteriaceae*	coliforms	*Staphylococcus aureus*	*Escherichia coli*	*Listeria* sp.	*Salmonella* sp.	Aflatoxin B_1
Loose									
Pig grower									
Batch 1	5.46 (0.28)	3.69 (0.37)	4.43 (0.11)	4.68 (0.31)	< 2.00	< 1.00	Absence	Absence	< 1.0 p.p.b.
Batch 2	4.72 (0.31)	3.30 (0.20)	3.77 (0.34)	4.26 (0.16)	< 2.00	< 1.00	Absence	Presence	< 1.0 p.p.b.
Batch 3	5.17 (0.43)	3.65 (0.24)	4.05 (0.26)	4.36 (0.36)	< 2.00	< 1.00	Absence	Absence	< 1.0 p.p.b.
Pig fattener									
Batch 1	5.43 (0.27)	4.07 (0.36)	4.74 (0.12)	4.89 (0.31)	< 2.00	2.17 (0.14)	Absence	Absence	< 1.0 p.p.b.
Batch 2	4.90 (0.39)	3.79 (0.15)	4.63 (0.16)	3.21 (0.23)	< 2.00	< 1.00	Absence	Absence	< 1.0 p.p.b.
Batch 3	5.07 (0.34)	3.57 (0.27)	4.56 (0.35)	3.64 (0.26)	< 2.00	< 1.00	Absence	Absence	< 1.0 p.p.b.
Sow lactation									
Batch 1	5.43 (0.23)	3.32 (0.17)	4.43 (0.14)	4.84 (0.20)	< 2.00	< 1.00	Absence	Absence	< 1.0 p.p.b.
Batch 2	5.04 (0.31)	4.04 (0.21)	4.56 (0.32)	3.59 (0.34)	< 2.00	< 1.00	Absence	Absence	< 1.0 p.p.b.
Batch 3	5.38 (0.28)	3.68 (0.47)	4.62 (0.39)	3.67 (0.31)	< 2.00	< 1.00	Absence	Absence	< 1.0 p.p.b.
Ewe lactation									
Batch 1	5.11 (0.20)	3.32 (0.10)	3.47 (0.25)	4.32 (0.14)	< 2.00	3.30 (0.21)	Absence	Absence	< 1.0 p.p.b.
Batch 2	4.44 (0.41)	3.39 (0.35)	3.95 (0.42)	< 1.00	< 2.00	< 1.00	Absence	Absence	< 1.0 ppb
Batch 3	4.85 (0.38)	3.36 (0.17)	3.23 (0.18)	< 1.00	< 2.00	< 1.00	Absence	Absence	< 1.0 p.p.b.
Calf fattener									
Batch 1	6.30 (0.28)	3.38 (0.14)	4.39 (0.34)	4.83 (0.48)	< 2.00	2.63 (0.35)	Absence	Absence	< 1.0 p.p.b.
Batch 2	5.07 (0.46)	3.74 (0.19)	4.60 (0.35)	4.63 (0.31)	< 2.00	< 1.00	Absence	Presence	< 1.0 p.p.b.
Batch 3	5.63 (0.36)	3.67 (0.28)	4.28 (0.37)	4.58 (0.28)	< 2.00	< 1.00	Absence	Absence	< 1.0 p.p.b.
Pelleted									
Pig grower									
Batch 1	3.70 (0.10)	< 2.00	2.80 (0.10)	2.60 (0.10)	< 2.00	< 1.00	Absence	Absence	< 1.0 p.p.b.
Batch 2	3.39 (0.30)	< 2.00	2.35 (0.14)	< 1.00	< 2.00	< 1.00	Presence	Absence	< 1.0 p.p.b.
Batch 3	3.61 (0.32)	< 2.00	< 1.00	< 1.00	< 2.00	< 1.00	Absence	Absence	< 1.0 p.p.b.
Pig fattener									
Batch 1	2.31 (0.23)	< 2.00	< 1.00	< 1.00	< 2.00	< 1.00	Absence	Absence	< 1.0 p.p.b.
Batch 2	2.97 (0.36)	< 2.00	2.67 (0.29)	< 1.00	< 2.00	< 1.00	Presence	Absence	< 1.0 p.p.b.
Batch 3	2.16 (0.13)	< 2.00	< 1.00	< 1.00	< 2.00	< 1.00	Absence	Absence	< 1.0 p.p.b.
Sow lactation									
Batch 1	2.60 (0.15)	< 2.00	< 1.00	< 1.00	< 2.00	< 1.00	Absence	Absence	< 1.0 p.p.b.
Batch 2	2.32 (0.21)	< 2.00	< 1.00	< 1.00	< 2.00	< 1.00	Absence	Absence	< 1.0 p.p.b.
Batch 3	2.25 (0.19)	< 2.00	< 1.00	< 1.00	< 2.00	< 1.00	Absence	Absence	< 1.0 p.p.b.
Ewe lactation									
Batch 1	2.95 (0.26)	< 2.00	< 1.00	< 1.00	< 2.00	< 1.00	Absence	Absence	< 1.0 p.p.b.
Batch 2	2.17 (0.37)	< 2.00	< 1.00	< 1.00	< 2.00	< 1.00	Absence	Absence	< 1.0 p.p.b.
Batch 3	2.23 (0.12)	< 2.00	< 1.00	< 1.00	< 2.00	< 1.00	Absence	Absence	< 1.0 p.p.b.
Calf fattener									
Batch 1	< 2.00	< 2.00	< 1.00	< 1.00	< 2.00	< 1.00	Absence	Absence	< 1.0 p.p.b.
Batch 2	< 2.00	< 2.00	< 1.00	< 1.00	< 2.00	< 1.00	Absence	Absence	< 1.0 p.p.b.
Batch 3	< 2.00	< 2.00	< 1.00	< 1.00	< 2.00	< 1.00	Absence	Absence	< 1.0 p.p.b.

Numbers of log CFU g^{-1}. All determinations were performed in triplicate. Standard deviation is given in parenthesis.
TBC, total bacterial count.

Given the recent adoption of Community legislation on Feed Hygiene (EU, 2005), the information from the present study will contribute to appropriate measures adopted by the state to cope with EU law on food safety (EU, 2002a), to develop a traceability procedure, and to establish an HACCP system, as well as a GMP programme for Greece and elsewhere.

References

ADAS (1986) *The Analysis of Agricultural Materials*. Agricultural Development Advisory Service, HMSO, London.

Aureli P., Fiorucci G.C., Caroli D., Marchiaro G., Navara O., Leone L., Salmaso S. (2000) An outbreak of febrile gastroenteritis associated with corn contaminated by *Listeria*

moncytogenes. *New England Journal of Medicine*, **342**, 1236–1241.

Coleman S.W., Moore J.E. (2003) Feed quality and animal performance. *Field Crop Research*, **30**, 17–29.

Coma J. (2003) *Salmonella* control in pork; effect of animal nutrition and feeding. *Pig News and Information*, **24**, 49–62.

Davies R.H., Wray C. (1997) Distribution of *Salmonella* contamination in ten animal feedmills. *Veterinary Microbiology*, **51**, 159–169.

Davis M.A., Hancock D.D., Rice D.H., Digiacomo R., Samadpour M., Besser T.E. (2003) Feedstuff as a vehicle of cattle exposure to *Escherichia coli* O157:H7 and *Salmonella enterica*. *Veterinary Microbiology*, **95**, 199–210.

EEC (1976) First Commission Directive 76/371/EEC of 1 March 1976 establishing community methods of sampling for the official control of feedingstuffs. *Official Journal of the European Communities*, **L102**, 1–7.

EU (2002a) Regulation (EC) No 178/2002 of the European Parliament and of the Council of 28 January 2002, laying down the general principles and requirements of food law, establishing the European Food Safety Authority and laying down procedures in matters of food safety. *Official Journal of the European Communities*, **L31**, 1–24.

EU (2002b) Directive 2002/32/EC of the European Parliament and of the Council of 7 May 2002 on undesirable substances in animal feed. *Official Journal of the European Communities*, **L140**, 10–21.

EU (2005) Regulation (EC) No 183/2005 of the European Parliament and of the Council of 12 January 2005, laying down requirements for feed. *Official Journal of the European Community*, **L35**, 1–22.

Fink-Gremmels J. (2006) Moulds and mycotoxins as undesirable substances in animal feed. In: *The Mycotoxin Fact Book: Food and Feed Topics* eds Barns D., Batnagar D., Van Egmond H.P., Van der Kamp J.W., Van Osenbruggen W.A., Visconti A. pp. 37–50, Wageningen Academic Publishers, the Netherlands.

Flachowsky G., Danicke S. (2005) From feed to safe food – contribution of animal nutrition to the safety of food. In: *New Developments in Food Policy, Control and Research* ed. Riley A.P. pp. 65–96, Nova Science Publications, New York.

Gohl B. (1981) *Tropical Feeds, FAO Animal Production and Health Series No 12*. Food and Agriculture Organization of the United Nations, Rome.

ISO (1998) Animal feedingstuffs: determination of aflatoxin B_1 content of mixed feedingstuffs – method using high performance liquid chromatography. ISO 14718.

Kan C.A., Meijer G.A.L. (2007) The risk of contamination of food with toxic substances present in animal feed. *Animal Feed Science and Technology*, **133**, 84–108.

Krytenburg D.S., Hancock D.D., Rice D.H., Besser T.E., Gan C.C., Gay J.M. (1998) A pilot survey of *Salmonella enterica* contamination of cattle feeds in the Pacific Northwestern USA. *Animal Feed Science and Technology*, **75**, 75–79.

Maciorowski K.G., Herrera P., Jones F.T., Pillai S.D., Ricke S.C. (2007) Effects on poultry and livestock of feed contamination with bacteria and fungi. *Animal Feed Science and Technology*, **133**, 109–136.

McDonald P., Edwards R.A., Greenhalgh J.F.D., Morgan C.A. (1995) *Animal Nutrition*, 5th Edition, pp. 411–432, Longman, Edinburg.

McIlroy S.G. (2001) Biosecurity programmes for *Salmonella* control. *Cahiers Options Méditerrannéennes*, **54**, 81–85.

Nightingale K.K., Schukken Y.H., Nightingale C.R., Fortes E.D., Ho A.J., Her Z., Grohn Y.T., McDonough P.L., Wiedman M. (2004) Ecology and transmission of *Listeria monocytogenes* infecting ruminants and in the farm environment. *Applied Environtmental Microbiology*, **41**, 179–183.

Petersen U., Flachowsky G. (2004) A positive list of feedstuffs as a contribution to feed safety: expectations, concepts and solutions. FAL Agricultural Research, Special Issue 271, Braunschweig, Germany.

Plumed-Ferrer C., Llopis M., Hyvonen P., von Wright A. (2004) Characterization of the microbial community and its changes in liquid piglet feed formulations. *Journal of the Science of Food and Agriculture*, **84**, 1315–1318.

Sales A.C., Yoshizawa T. (2006) *Aspergillus* Section *Flavi* and aflatoxins in dusts generated by agricultural processing facilities in the Philippine. *Journal of the Science of Food and Agriculture*, **86**, 2534–2542.

Smulders F.J.M., Kainz R., Weijtens M.J.B.M. (2006) The new EU legislation on food control and how veterinarians fit in. In: *Towards a Risk Based Chain Control* ed. Smulder F.J.M. pp. 201–226, Wageningen Academic Publishers, the Netherlands.

Sofos J.N. (2006) Field data availability and needs for use in microbiological risk assessment. In: *Towards a Risk Based Chain Control* ed Smulder F.J.M. pp. 57–74, Wageningen Academic Publishers, the Netherlands.

Vlachou S., Zoiopoulos P.E., Drosinos E.H. (2004) Assessment of some hygienic parameters of animal feeds in Greece. *Animal Feed Science and Technology*, **117**, 331–337.

