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Research Papers

Prevalence of mycotoxins in feedstuffs and feed surveyed worldwide in 2009 and 2010

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Summary. Mycotoxins are becoming an increasingly important topic in both human and animal fields. With the improvement of analytical methods it is possible to identify a great number of known and unknown toxic metabolites. However, even for the most well studied mycotoxins, namely aflatoxins, deoxynivalenol, zearalenone, fumonisins and ochratoxin A, several questions remain unanswered. In which commodities are they most commonly found and at which levels? Which worldwide regions are more prone to mycotoxin occurrence and how severe is the contamination? In an attempt to answer these questions, a worldwide mycotoxin survey has been been carried out. A total of 6,058 feedstuffs and finished feed samples were analyzed between January 2009 and December 2010 for the presence of the aforementioned mycotoxins. 1,695 samples were analyzed by Enzyme Linked Immunosorbent Assay (ELISA) and 4,363 samples were analyzed by High Performance Liquid Chromatography (HPLC). This paper presents the results of these analyses from a geographical point of view (by world region) and by commodity type, separated by analytical method. The outcome is clear and mycotoxins are reported to be ubiquitously present as 24, 21, 65, 48 and 17% of analyzed samples tested positive for aflatoxins, deoxynivalenol, zearalenone, fumonisins and ochratoxin A, respectively in the case of ELISA-analyzed samples and 31, 44, 50, 56 and 27% of HPLC-analyzed samples tested positive for the same mycotoxins.

Key words: aflatoxins, trichothecenes, fumonisins, zearalenone, mycotoxins.

Introduction

The ubiquitous presence of mycotoxins is an undeniable fact. The types of mycotoxins present in an agricultural commodity depend on several factors. In the field, where most of the mycotoxin production takes place, the environmental conditions during ripening and the agricultural practices are the most decisive factors (Blandino *et al.*, 2009). After harvest, conditions of moisture and temperature have a major impact on fungal growth and mycotoxin production, which dictate major differences in contamination between the so-called developed or developing countries, as in the latter it is not unusual for grain to become moldy during storage (Bryden, 2009). Up until now, the European Feed Safety Authority (EFSA) has published scientific reports on aflatoxins, ochratoxin A, fumonisins, zearalenone and deoxynivalenol. However, as regards to animal feed, aflatoxin B_1 is still the only legislated mycotoxin. Directive 2002/32/EC of the European Parliament and of the Council of 7 May 2002 on undesirable substances in animal feed sets maximum levels to limit as much as possible the presence of aflatoxin B_1 in animal feed put into circulation within the European Union (EU) (Directive, 2002). As for deoxynivalenol, zearalenone, ochratoxin A, T-2 and HT-2 and fumonisins, the European Commission has released a document with guidance values on the presence of these contaminants in products intended for animal feeding (Commission Recommendation, 2006). Therefore, such mycotoxins seem to be the most relevant in regards to feed contamination and economic impact in the animal industry.

Published scientific studies and surveys regarding the existence of such contaminants are usually

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limited to a single commodity or to a single mycotoxin, to a country, to a short period of time and include a small number of samples, more often than not analyzed by ELISA methods rather than by HPLC. Many of them actually focus on foods intended for human consumption. As for commodities used for animal rations, extensive data are not available.

With the objective of further understanding the worldwide prevalence of mycotoxins in feedstuffs and feeds, a survey was done between January 2009 and December 2010 in which a total of 6,058 samples were analyzed for the presence of aflatoxins (Afla), deoxynivalenol (DON), zearalenone (ZON), fumonisins (FUM) and ochratoxin A (OTA) (1,695 samples were analyzed by ELISA and 4,363 were analyzed by HPLC). This paper shows the outcome of the more than 20,417 analyses performed to commodities and rations intended for animal consumption. Results are grouped by geographical region, by commodity type and their distribution is presented by contamination ranges. All data are separated according to analytical methods (ELISA or HPLC) used.

Materials and methods

Analytical samples

A total of 6,058 grain, feed and other feed commodities samples were sourced directly from animal farms or animal feed production sites in diverse regions. The origin of the samples was diverse, covering all worldwide regions. North American samples refer to those which were sourced in the United Stated of America (U.S.) and Canada. Brazilian (99%) and Argentinean samples are gathered in the group named South America. 5 samples were sourced in Guatemala and are grouped in Central America. Likewise, Europe was divided into regions: Northern Europe (samples from Norway, Sweden, Finland, Denmark, Lithuania, Russia and United Kingdom), Central Europe (samples from Austria, Belgium, Germany, France, Hungary, Romania, Slovakia, Slovenia, Poland, Ukraine, Czech Republic and Croatia) and Southern Europe (samples from Greece, Italy, Portugal, Spain and Turkey). The group named 'Middle East and North Africa' gathers samples sourced in Algeria, Israel, Lebanon, Saudi Arabia, Sudan, Syria, United Arabic Emirates and Yemen. South Africa, Nigeria, Kenya and Ghana are combined in the group Africa. The North Asia group comprises samples sourced in China, Japan, Korea and Taiwan; the South-East Asian group reflects the results of samples from Malaysia, Philippines, Thailand, Vietnam and Indonesia and the South Asian group gathers samples from Sri Lanka, Pakistan, Bangladesh and India. Finally, samples from New Zealand and Australia are gathered in the Oceania group. In terms of commodities and due to their diverse nature, samples were divided into groups, namely 'maize', 'soybean and soybean meal', 'wheat and wheat bran', 'maize gluten meal (CGM)', 'rice and rice bran', 'dried distillers grains with solubles (DDGS)', 'straw', 'silage', 'finished feed', 'barley' and 'other commodities'. The latter includes different commodities for which the number of samples was not significant enough to display in a separate group; specifically grass and alfalfa silage, cotton seed, sunflower meal, sorghum and fish meal. Only single commodities were analyzed by ELISA. More complex matrixes which could interfere with the ELISA method such as CGM, DDGS, finished feed, silage and straw were analyzed by HPLC. Besides the nature of the samples, also the geographical availability of analytical methods determined that certain feedstuffs were analyzed by ELISA rather than HPLC. ELISA was performed in samples sourced in North America (15% of ELISA-analyzed samples) and Europe (85% of the samples), exclusively. HPLC was performed in samples from Asia (56% of HPLC-analyzed samples), North America (3.9%), South America (15.5%), Europe (16.6%), Middle East (3.7%) and Africa (4.3%).

Good sampling methods (Richard, 2000) were explained and sample providers were advised to follow them; however, as in the case of any analyses performed by independent laboratories, analytical personnel and/or laboratory staff were not involved and therefore were not able to influence any part of this procedure. Samples received in the lab weighed approximately 1 kg. Generally, collection of a representative sample should involve the collection of several small randomly selected samples ("incremental samples") from the whole lot to form what is known as "lot sample". After grinding and/or homogenizing the full lot sample, a subsample was taken for the actual analytical process ("analytical sample"). For dry and low-fat containing samples such as various raw cereals, a ROMER Series II® subsampling mill (Romer Labs® Diagnostic GmbH, Tulln, Austria) was used. For all other commodities and already ground samples, kitchen blenders or other adequate instruments were used for homogenization.

A choice could be made regarding the mycotoxins to be analyzed for, either "full toxin screen", which covered aflatoxins (referred to as Afla, a sum of aflatoxin B_1, aflatoxin B_2, aflatoxin G_1 and aflatoxin G_2), deoxynivalenol (DON), zearalenone (ZON), fumonisins (referred to as FUM, a sum of fumonisin B_1 and fumonisin B_2), and ochratoxin A (OTA), or analyses of selected mycotoxins. For this reason, the number of analyzed samples in certain regions is sometimes different depending on the specific mycotoxin. The origin (name and location of submitter) of samples was kept strictly confidential; analytical certificates were submitted only to the originators of samples.

For the purpose of data analysis, non-detect levels are based on the detection limits (LOD) of the test method for each toxin (Table 1).

Table 1. Limits of detections (LOD) of methods applied.

Mycotoxin	HPLC ppb	ELISA ppb
Afla (sum of AfB_1, AfB_2, AfG_1, AfG_2)	-	1
AfB_1, AfB_2, AfG_1, AfG_2	0.3, 0.1, 0.1, 0.1	-
ZON	10	40
DON	50	250
FB_1,FB_2	25, 25	-
FUM (sum of FB_1 and FB_2)	-	250
OTA	0.2	2

Mycotoxin analysis

Mycotoxin analyses were carried out as published and thoroughly described in Griessler *et al.* (2010) by the same accredited analytical laboratory. Extraction was performed with a rotary shaker (GFL, Burgwedel, Germany) after adding water or mixtures of organic solvents to the weighed sample aliquot.

Enzyme Linked Immunosorbent Assay – (ELISA)

ELISA (Enzyme linked immunosorbent assay) analyzes were performed with a commercially available test kit (AgraQuant®Assay, Romer Labs®), applicable to most cereal raw commodities. Chromatographic tests were suitable for all commodities.

The AgraQuant® Assay is a direct competitive ELISA. Mycotoxins were extracted from a ground sample with a specific solvent. The extracted sample and enzyme-conjugated mycotoxin were mixed and added to the antibody-coated microwell. Mycotoxins in samples and control standards were allowed to compete with enzyme-conjugated mycotoxins for the antibody binding sites. After a washing step, an enzyme substrate was added and blue colour developed. The intensity of the color was inversely proportional to the concentration of mycotoxins in the sample or standard. A stop solution was then added which changed the color from blue to yellow. The microwells were measured optically by a microplate reader with an absorbance filter. The optical densities of the samples were compared to the OD's of the standards and an interpretative result was determined.

In the described study AgraQuant® Total Aflatoxin Assay 1/20, AgraQuant® Zearalenone Assay 40/100, AgraQuant® Deoxynivalenol Assay 0.25/5.0, AgraQuant® Total Fumonisin Assay 0.25/5.0 and AgraQuant® Ochratoxin 2/40 were used. The first number in the test kits name refers to the limit of quantification [$\mu g\ kg^{-1}$ or ppb], the second number to the upper limit of the working range [$\mu g\ kg^{-1}$ or ppb] of the respective kit.

The AgraQuant® Total Aflatoxin Assay was validated for maize, barley and other commodities. Afla was extracted from 20 g representative samples by shaking with 100 mL of 70% methanol for 1 hour at 180 rpm (rotations per minute) on a GFL shaker. After performing the ELISA test according to the described procedure, the micro wells were measured by using an absorbance filter of 450 nm and a differential filter of 630 nm.

The AgraQuant® ZON Assay was validated by the manufacturer for the use in maize, wheat, barley and other commodities. ZON was extracted from 20 g representative samples by shaking with 100 mL of 70% methanol for 1 hour at 180 rpm (rotations per minute) on a GFL shaker. After performing the ELISA test according to the described procedure, the micro wells were measured by using an absorbance filter of 450 nm and a differential filter of 630 nm.

The AgraQuant® DON Assay was validated by the manufacturer for the use in maize, wheat, barley and other commodities. 20 g of representative sample were weighed into a glass flask; 100 mL of distilled water were added. The sample was extracted by shaking for 1 hour at 180 rpm (rotations per minute) on a GFL shaker. After performing the ELISA test according to described procedure, micro wells were measured by using an absorbance filter of 450 nm (OD450).

The AgraQuant® Total Fumonisin Assay was validated by the manufacturer for maize, wheat, rice and other commodities. For extraction, 100 mL of 70% methanol were added to 20 g of the sample and shaken for 1 hour as described for Afla, ZON or DON previously. After performing the test according to the manufacturer's guidelines, the micro wells were measured by using an absorbance filter of 450 nm and a differential filter of 630 nm.

The AgraQuant® Ochratoxin Assay was validated for wheat, maize, barley and other commodities. OTA was extracted from 20 g representative samples by shaking with 100 mL of 70% methanol for 1 hour as described for Afla, ZON or DON previously. After performing the ELISA test according to the described procedure, the micro wells were measured by using an absorbance filter of 450 nm and a differential filter of 630 nm.

High performance liquid chromatography (HPLC)

All chromatographic methods applied are based on high performance liquid chromatography (HPLC), using in-house methods as well as official methods such as European standard methods if available. All chromatographic methods required sample clean-up prior to detection. Purification was accomplished either by using immune-affinity clean-up columns (all Romer Labs®) or MycoSep® clean-up columns (Romer Labs®). HPLC analyses were performed with 1100 and 1200 series HPLC systems, each equipped with a UV detector (G1314B) and a Fluorescence detector (G1321A), or a mass spectrometer (G1946D) from Agilent Technologies (Waldbronn, Germany). Depending on the optical properties of the mycotoxins of interest, several detectors can be used: e.g. the mycotoxins such as OTA or ZON and Afla after derivatization show a natural fluorescence, thus they were detected with high sensitivity by use of fluorescence detectors. Others such as type B trichothecenes (e.g. DON) absorb UV-light of a certain wavelength and were measured by a UV-detector. For those mycotoxins that do not emit fluorescence light or absorb UV-light (such as FUM) a mass spectrometer (single quadrupole) was used for determination.

Results and discussion

Tables 2 and 3 give an overview of the survey results. Table 2 depicts results obtained after analyses of samples by ELISA and Table 3 shows the results of HPLC analyses. The number of samples analyzed by HPLC was higher than those analyzed by ELISA.

Results in Table 2 show that 24, 21, 65, 48 and 17% of the samples tested positive for contamination with Afla, ZON, DON, FUM and OTA, respectively. Results obtained from the HPLC analyses to other

Table 2. Overview of the survey (ELISA).

Global results 2009 and 2010	Afla	ZON	DON	FUM	OTA
Number of tests	115	1,189	1,638	197	75
Percent positive (%)[a]	24	21	65	48	17
Average (ppb)[b]	1	24	651	804	6
Median of positive (ppb)[c]	4.1	0.0	577.5	520.0	6.0
1st quartile of positive (ppb)[d]	1.5	58.0	379.8	380.0	3.4
3rd quartile of positive (ppb)[d]	7.3	140.0	1,069.0	1,140.0	10.8
Maximum (ppb)	26	1,014	14,137	22,900	331
Commodity found	Corn	Corn	Barley	Corn	Wheat
Country of origin	USA	USA	Austria	USA	Austria

[a] Percent positive refers to results above the limit of detection of the method applied.
[b] Average corresponds to the average of all results, including results below the limit of detection, which were set as zero for the calculations.
[c] Median of positive excludes results below the limit of detection.
[d] 1st and the 3rd quartile of positive excludes results below the limit of detection.

Table 3. Overview of the survey (HPLC).

Global results 2009 and 2010	Afla	ZON	DON	FUM	OTA
Number of tests	3,570	3,786	3,741	3,563	2,543
Percent positive (%)[a]	31	44	50	56	27
Average (ppb)[b]	20	115	553	1,146	3
Median of positive (ppb)[c]	11.0	0.0	421.0	993.0	2.7
1st quartile of positive (ppb)[d]	3.0	41.0	215.0	448.5	1.2
3rd quartile of positive (ppb)[d]	43.0	225.0	978.8	2,343.5	6.2
Maximum (ppb)	6,105	16,712	49,000	53,700	1,582
Commodity found	Corn	CGM	Wheat	Corn	Finished Feed
Country of origin	Vietnam	China	Austria	Brazil	Pakistan

[a, b, c, d] See Table 2.

samples revealed that the most commonly occurring mycotoxin was FUM with 56% of positive samples, followed by DON, ZON, Afla and OTA with 50, 44, 31 and 27% of positive samples, respectively. These results reflect the trend observed in the different regions where the different methods were applied. For example, ELISA was only used in samples from Europe and U.S., whereas HPLC was used, besides these regions, mainly in samples from Asia and Brazil which account for a great proportion of the survey samples. The high prevalence of FUM in Brazil (Table 5d) was probably a factor leading to an increased percentage of FUM occurrence in the overall HPLC results. On the other hand, DON is very prevalent in samples from North America (Table 5a and 5b) and Europe (Table 6a, 6b, 6c) which made DON the most commonly occurring mycotoxin in samples analyzed by ELISA.

Results by geographic region

Interesting differences were obtained for the different regions; within the same region, sub-regions also differed considerably. Table 4 presents survey results for the Asian region obtained by HPLC.

In North Asia, the most prevalent mycotoxins are those produced by *Fusarium* fungi, namely DON, ZON and FUM. DON was present in 70% of tested samples at an average of 691 ppb. These results are in accordance with those of Binder *et al.* (2007) where DON, ZON and FUM were the most prevalent mycotoxins, found in 71%, 47% and 49% of analyzed samples. In South East Asia, the most prevalent mycotoxins were Afla and FUM, present in 60% of analyzed samples at an average level of 43 ppb and 787 ppb, respectively. ZON and DON were present in 47% and 36% of tested samples, at an average of 63 ppb and 292 ppb, respectively. Likewise, Yamashita *et al.* (1995) reported the simultaneous occurrence of Afla, FUM and *Fusarium* toxins in maize from Philippines, Thailand and Indonesia. Generally produced by the genera *Aspergillus* and *Penicillium* in subtropical and warm temperate climates (CAST, 2003), Afla and OTA were most prevalent in countries such as India, Pakistan and Bangladesh where 83% and 67% of analyzed samples tested positive for these mycotoxins, respectively. Nonetheless, as reported by Summerell *et al.* (2001), *Fusarium* is a large and complex genus with species adapted to a wide range of habitats. Results of the present survey show that the importance of fusariotoxins in this sub-region must not be disregarded as FUM, ZON and DON were present in 62%, 35% and 24% of tested samples. Within the Asia-Pacific region, Australia and New Zealand showed a different mycotoxin pattern, with oftentimes a lower prevalence of mycotoxins when in comparison with neighbor countries.

Table 5 presents survey results from North and South America obtained both by ELISA (Table 5a) and HPLC (Table 5b to 5d). North America's concern with mycotoxins such as DON and ZON is widely known, especially after 2009 crop, acknowledged in

Table 4. Survey results by geographic region (Asian region, HPLC).

Region/HPLC	Afla	ZON	DON	FUM	OTA
North Asia (includes China, Taiwan, Korea and Japan)					
Number of tests	1,148	1,176	1,196	1,178	1,133
Percent positive (%)	12	57	70	43	20
Average (ppb)	8	219	691	797	1
Median of positive (ppb)	9.9	153.0	501.0	946.0	2.7
1st quartile of positive (ppb)	3.0	68.1	252.8	334.0	1.4
3rd quartile of positive (ppb)	30.5	392.5	1,050.3	2,223	6.9
Maximum (ppb)	4,687	16,712	19,141	14,654	60
South East Asia (includes Malaysia, Philippines, Thailand, Vietnam and Indonesia)					
Number of tests	748	726	615	748	617
Percent positive (%)	60	47	36	60	33
Average (ppb)	43	63	292	787	2
Median of positive (ppb)	16.0	49.0	219.5	727.5	2.0
1st quartile of positive (ppb)	5.0	35.0	111.0	388.5	0.8
3rd quartile of positive (ppb)	47.0	92.0	569.0	1,332.0	4.1
Maximum (ppb)	6,105	2,721	19,096	32,510	80
South Asia (includes India, Pakistan and Bangladesh)					
Number of tests	188	185	174	188	186
Percent positive (%)	83	35	24	62	67
Average (ppb)	90	32	61	380	15
Median of positive (ppb)	38.0	53.0	139.0	443.0	4.1
1st quartile of positive (ppb)	12.5	33.0	85.5	216.0	1.9
3rd quartile of positive (ppb)	120.0	81.0	280.0	674.0	8.5
Maximum (ppb)	2,454	1,099	1,330	6,196	1,582
Oceania (includes Australia and New Zealand)					
Number of tests	235	234	233	231	229
Percent positive (%)	10	18	24	8	14
Average (ppb)	1	50	94	109	1
Median of positive (ppb)	6.9	54.0	249.0	1,027.0	3.2
1st quartile of positive (ppb)	2.0	38.0	111.5	119.0	1.9
3rd quartile of positive (ppb)	11.6	312.0	435.0	2,122.0	7.8
Maximum (ppb)	51	3,909	2,577	5,438	111.2

the feed industry as a year of exceptionally high contamination. DON was present in 88% of tested samples at average levels of 876 ppb in the case of samples tested by ELISA. The trend observed is not very different to that observed in HPLC-tested samples where DON was also the most prevalent mycotoxin,

Table 5. Survey results by geographic region (North and South America region, ELISA and HPLC).

Region/ELISA	Afla	ZON	DON	FUM	OTA
a - North America (U.S. and Canada)					
Number of tests	59	240	254	175	0
Percent positive (%)	20	41	88	50	-
Average (ppb)	2	58	876	725	-
Median of positive (ppb)	7.4	97.5	710.0	515.0	-
1st quartile of positive (ppb)	5.2	72.6	500.0	380.0	-
3rd quartile of positive (ppb)	11.7	162.8	1,230.0	1,020.0	-
Maximum (ppb)	26	1,014	5,210	22,900	-

Region/HPLC	Afla	ZON	DON	FUM	OTA
b - North America (U.S. and Canada)					
Number of tests	129	170	171	153	110
Percent positive (%)	12	53	78	57	20
Average (ppb)	8	271	1,947	902	1
Median of positive (ppb)	2.0	140.5	1,064.0	1,000.0	2.1
1st quartile of positive (ppb)	2.0	77.0	438.3	464.0	1.4
3rd quartile of positive (ppb)	16.9	344.0	2,275.0	1,778.5	4.5
Maximum (ppb)	831	8,952	29,300	11,381	14
c - Central America					
Number of tests	5	2	5	5	5
Percent positive (%)	20	0	80	100	0
Average (ppb)	2	0	237	1,030	0
Median of positive (ppb)	10.4	-	281.0	989.0	-
1st quartile of positive (ppb)	10.4	-	259.8	822.0	-
3rd quartile of positive (ppb)	10.4	-	317.0	1,144.0	-
Maximum (ppb)	10.4	-	395	1,746	-
d - South America (Brazil)					
Number of tests	637	409	370	648	107
Percent positive (%)	31	54	17	88	7
Average (ppb)	3	111	51	3,121	9
Median of positive (ppb)	2.2	86.5	173.5	2,226.0	77.5
1st quartile of positive (ppb)	1.0	35.3	140.0	972.5	0.95
3rd quartile of positive (ppb)	5.9	213.5	269.7	4,420.0	215.0
Maximum (ppb)	342	5,930	2,520	53,700	355

present in 78% of samples at average levels of 1,947 ppb. The number of samples tested in Central America was too low to draw conclusions regarding mycotoxin prevalence in this region. Samples sourced from Brazil account for 99% of the South American sub-group whereas only 5 samples were sourced from Argentina. In this sub-group (Table 5d) FUM were the major contaminants (88% positive samples) at average levels of 2,226 ppb. ZON was also found to be very prevalent (54% positive samples; average 111 ppb). Similar results were obtained in a survey done in freshly harvested Brazilian maize (Camargos *et al.*, 2001) where FUM were found in all the 110 samples tested. In the same study Afla were present in 55% of analyzed samples.

Results obtained by ELISA for Europe and Middle East are shown in Table 6 (6a to 6d) and those obtained by HPLC for the same regions and Africa are shown in Table 6e to 6i.

In North Europe, ELISA results show the prevalence of DON (72% positive; average 644 ppb) followed by OTA (18% positive, average of 1 ppb). However, HPLC results show a prevalence of OTA in this region (54% positive, average of 3 ppb). This is a quite interesting finding as this mycotoxin is commonly found in tropical regions (as observed in results of South Asia, where OTA was found in 67% of samples at average levels of 15 ppb). The reason for this relies on the fact that OTA is mainly produced by some strains of *Aspergillus ochraceus* (which optimum for OTA production is 25–31ºC) and *Penicillium verrucosum* (which optimum temperature for OTA production is 20–25ºC), therefore it is reasonable to hypothesize that the OTA found in this region was produced by different fungi than those found in South Asia. Actually, as reviewed by Denli and Perez (2010), the highest amounts of this mycotoxin have been reported in Northern Europe and North America. The most prevalent mycotoxin in Central Europe was found to be DON (both in ELISA and HPLC tested samples). Actually, the highest DON levels of this survey were obtained on a barley (14,137 ppb) and on a wheat (49,000 ppb) sample from Austria. Samples sourced from Southern European countries show the increasing prevalence of Afla and FUM. Even if percentage values in terms of occurrence of mycotoxins in samples analyzed by ELISA and HPLC do not totally coincide, average levels of mycotoxins do not differ greatly, except for DON, for which average levels found by ELISA tests are higher (646 ppb) than those found by HPLC (275 ppb). Aksoy *et al.* (2009) analyzed by ELISA the occurrence of aflatoxin B_1, T-2 toxin and zearalenone in forty compound animal feed samples collected in Turkey and these mycotoxins were reported to be present in 95, 65 and 87.5% of tested samples, respectively. Only two samples sourced in Middle East were analyzed by ELISA, therefore no conclusions can be drawn from these results. Samples from this region analyzed by HPLC show a prevalence of FUM with 44% of samples testing positive for this mycotoxin; however, other mycotoxins such as DON and OTA were also present in 38% and 33% of analyzed samples and ZON and Afla tested positive in 17% and 14% of these. A study published in 2000 reported the prevalence of FUM in maize from the same region (Iran) with levels reaching 3,980 ppb (Shepard *et al.*, 2000). In the African countries surveyed it was also interesting to see that FUM and DON were the most prevalent mycotoxins, present in 71% and 70% of samples, respectively (average levels of 855 ppb and 745 ppb, respectively). When single countries are accounted for it is possible to see that different countries influence this result in distinctive ways. The most prevalent mycotoxins in South Africa are DON and FUM, whereas countries like Ghana, Kenya and Nigeria have higher prevalence of Afla (Rodrigues *et al.*, 2011). In a previous survey done in South Africa, six brands of agricultural feeds, eleven different raw ingredients used in animal feed production and six brands of commercially available pet food were tested by HPLC for the presence of aflatoxins, fumonisin B_1 and zearalenone (Mngadi *et al.*, 2008). Among the tested samples, 74% tested positive for aflatoxins while fumonisin B_1 was present in 26% and zearalenone in 13% of samples.

Results by commodity

Regarding commodities (Table 7a and Table 7b), maize was the most widely tested commodity. FUM and DON were found to be the mycotoxins of higher prevalence. DON was more prevalent in ELISA-tested samples (78% of positive) probably due to the fact that these are sourced from European or North American countries, where this mycotoxin is more prevalent. In HPLC-tested samples, the majority originated in Asian and South American regions, and FUM was shown to be more prevalent (83% positive). Maize by-products contained ZON, DON and FUM at average levels of 1,848 ppb, 1,705 ppb and

Table 6. Survey results by geographic region (Europe and Middle East regions), determined by ELISA and by HPLC.

Region/ELISA	Afla	ZON	DON	FUM	OTA
a - North Europe (Norway, Sweden, Finland, Denmark, Lithuania, Russia and United Kingdom)					
Number of tests	3	122	123	0	17
Percent positive (%)	0	2	72	-	18
Average (ppb)	0	1	644	-	1
Median of positive (ppb)	-	61.0	520.0	-	6.0
1st quartile of positive (ppb)	-	59.0	378.0	-	5.7
3rd quartile of positive (ppb)	-	63.0	823.0	-	7.8
Maximum (ppb)	0	65	10,440	-	9.6
b - Central Europe (Austria, Belgium, Germany, France, Hungary, Romania, Slovakia, Slovenia, Poland, Ukraine,Czech Republic and Croatia)					
Number of tests	27	795	1,220	14	48
Percent positive (%)	22	17	59	36	21
Average (ppb)	0	17	607	925	9
Median of positive (ppb)	1.6	78.0	533.0	270.0	7.7
1st quartile of positive (ppb)	1.3	48.7	366.0	43.9	2.9
3rd quartile of positive (ppb)	1.9	140.0	1,025.8	6,770.0	17.5
Maximum (ppb)	2	522	14,137	7,680	331
c - South Europe (Italy, Greece, Portugal, Spain and Turkey)					
Number of tests	24	30	39	6	8
Percent positive (%)	42	20	67	33	0
Average (ppb)	2	21	646	3,052	0
Median of positive (ppb)	1.5	96.0	673.0	9,155.0	-
1st quartile of positive (ppb)	1.3	80.9	364.0	8,207.5	-
3rd quartile of positive (ppb)	3.7	109.6	1,285.8	10,102.5	-
Maximum (ppb)	18	166	3,505	11,050	0
d - Middle East and North Africa (Algeria, Israel, Lebanon, Saudi Arabia, Sudan, Syria, United Arabic Emirates and Yemen)					
Number of tests	2	2	2	2	2
Percent positive (%)	0	50	0	0	0
Average (ppb)	0	10	0	0	0
Median of positive (ppb)	-	20.0	-	-	-
1st quartile of positive (ppb)	-	20.0	-	-	-
3rd quartile of positive (ppb)	-	20.0	-	-	-
Maximum (ppb)	0	20	0	0	0

(*Continued*)

Table 6. *Continues.*

Region/HPLC	Afla	ZON	DON	FUM	OTA
e - North Europe (Norway, Sweden, Finland, Denmark, Lithuania, Russia and United Kingdom)					
Number of tests	12	41	41	9	13
Percent positive (%)	0	7	37	22	54
Average (ppb)	0	3	88	51	3
Median of positive (ppb)	-	34.0	209.0	229.5	3.0
1st quartile of positive (ppb)	-	31.0	165.0	226.3	2.1
3rd quartile of positive (ppb)	-	37.5	272.0	232.8	6.8
Maximum (ppb)	-	41	536	236	11
f - Central Europe (Austria, Belgium, Germany, France, Hungary, Romania, Slovakia, Slovenia, Poland, Ukraine,Czech Republic and Croatia)					
Number of tests	79	453	512	51	80
Percent positive (%)	4	33	53	29	46
Average (ppb)	0	37	968	478	1
Median of positive (ppb)	1.8	42.5	503.5	530.0	2.5
1st quartile of positive (ppb)	1.7	25.0	246.5	213.5	1.2
3rd quartile of positive (ppb)	2.4	99.0	1,221.0	2,236.0	4.4
Maximum (ppb)	3	1045	49,000	5,489	12
g - South Europe (Italy, Greece, Portugal, Spain and Turkey)					
Number of tests	54	76	101	34	26
Percent positive (%)	26	17	56	91	38
Average (ppb)	3	13	275	2,347	0
Median of positive (ppb)	3.3	54.0	421.0	2,339.0	0.7
1st quartile of positive (ppb)	1.4	37.0	268.0	1,525.5	0.6
3rd quartile of positive (ppb)	5.3	75.0	624.0	3,462.0	0.9
Maximum (ppb)	103	237	2,160	7,714	2.8
h - Middle East (Algeria, Israel, Lebanon, Saudi Arabia, Sudan, Syria, United Arabic Emirates and Yemen)					
Number of tests	158	154	152	147	27
Percent positive (%)	14	17	38	44	33
Average (ppb)	8	14	153	280	4
Median of positive (ppb)	4.8	59.0	277.0	380.0	3.4
1st quartile of positive (ppb)	2.3	43.0	213.0	260.8	2.1
3rd quartile of positive (ppb)	34.9	91.5	413.8	865.8	18.3
Maximum (ppb)	388	392	1,620	2948	31.3

(*Continued*)

5,871 ppb (maize gluten meal) and 261 ppb, 2,883 ppb and 1,150 ppb (DDGS), respectively. Similarly to this survey, a large-scale survey was done involving 235 distillers dried grains with soluble (DDGS) samples sourced from the U.S. and Taiwan (Zhang *et al*., 2009). Although no samples were reported to surpass the

Table 6. *Continues.*

Region/HPLC	Afla	ZON	DON	FUM	OTA
i- Africa (South Africa, Nigeria, Kenya and Ghana)					
Number of tests	177	160	171	171	10
Percent positive (%)	47	35	70	71	60
Average (ppb)	42	25	745	855	6
Median of positive (ppb)	44.7	42.5	451.0	961.5	12.0
1st quartile of positive (ppb)	12.0	33.0	334.0	595.3	12.0
3rd quartile of positive (ppb)	91.7	73.3	1,048.0	1,466.0	12.0
Maximum (ppb)	556.4	310	11,022	10,485	12

Table 7a. Survey results by commodity, determined by ELISA.

Commodity/ELISA	Afla	ZON	DON	FUM	OTA
Corn					
Number of tests	50	394	459	157	12
Percent positive (%)	8	32	78	45	8
Average (ppb)	1	41	742	813	1
Median of positive (ppb)	11.2	85.2	626.0	500.0	10.8
1st quartile of positive (ppb)	3.7	59.7	420.0	355.0	10.8
3rd quartile of positive (ppb)	20.1	154.3	1,055.5	1,000.0	10.8
Maximum (ppb)	26	1,014	7,591	22,900	11
Soybean/Soybean meal					
Number of tests	7	15	24	1	4
Percent positive (%)	57	0	42	0	25
Average (ppb)	1	0	202	0	5
Median of positive (ppb)	1.3	-	434.0	-	21.4
1st quartile of positive (ppb)	1.3	-	348.8	-	21.4
3rd quartile of positive (ppb)	1.4	-	487.0	-	21.4
Maximum (ppb)	2	0	908	0	21.4
Wheat/bran					
Number of tests	15	208	325	3	20
Percent positive (%)	47	7	58	0	20
Median of positive (ppb)	2.0	53.0	494.5	-	5.7
1st quartile of positive (ppb)	1.4	46.5	360.0	-	4.8
3rd quartile of positive (ppb)	3.1	72.0	914.3	-	87.3
Maximum (ppb)	6	145	4,557	0	331

Table 7b. Survey results by commodity, determined by HPLC.

Commodity/HPLC	Afla	ZON	DON	FUM	OTA
Corn					
Number of tests	1,077	979	878	1,110	557
Percent positive (%)	38	39	53	83	11
Average (ppb)	38	121	634	2,431	3
Median of positive (ppb)	11.0	121.0	474.0	1,533.5	3.1
1st quartile of positive (ppb)	2.1	50.0	238.0	684.5	1.3
3rd quartile of positive (ppb)	62.0	287.8	1,185.0	3,671.5	12.5
Maximum (ppb)	6,105	7,422	26,121	53,700	355
Corn gluten meal					
Number of tests	30	30	30	29	30
Percent positive (%)	50	93	87	100	67
Average (ppb)	37	1,848	1,705	5,871	6
Median of positive (ppb)	5.0	1,193.0	380.5	2,437.0	5.4
1st quartile of positive (ppb)	3.0	495.5	150.0	967.0	2.3
3rd quartile of positive (ppb)	24.7	2,350.8	2,312.0	7,714.0	8.1
Maximum (ppb)	542	16,712	11,836	32,510	59.7
Soybean/soybean meal					
Number of tests	159	156	159	157	122
Percent positive (%)	18	14	30	6	20
Average (ppb)	1	13	62	27	1
Median of positive (ppb)	2.0	36	157.0	311.0	1.9
1st quartile of positive (ppb)	1.0	31.0	98.8	238.0	1.0
3rd quartile of positive (ppb)	4.0	51.8	222.0	321.0	4.9
Maximum (ppb)	42	807	1,019	2,035	23
Wheat/bran					
Number of tests	177	184	191	175	124
Percent positive (%)	5	34	53	9	21
Average (ppb)	0	43	1,400	28	1
Median of positive (ppb)	2.6	53.0	398.5	254.0	2.1
1st quartile of positive (ppb)	1.8	38.2	167.0	119.8	1.1
3rd quartile of positive (ppb)	7.0	204.0	1,515.3	407.3	4.9
Maximum (ppb)	20	513	49,000	874	30
DDGS					
Number of tests	89	101	105	93	91
Percent positive (%)	9	85	90	81	35
Average (ppb)	2	261	2,883	1,150	2

(*Continued*)

Table 7b. *Continues.*

Commodity/HPLC	Afla	ZON	DON	FUM	OTA
Median of positive (ppb)	6.3	181.5	2,422.0	798.0	2.9
1st quartile of positive (ppb)	1.1	95.4	1,067.8	363.5	1.4
3rd quartile of positive (ppb)	36.5	326.5	4,708.3	1,576.5	5.9
Maximum (ppb)	44	2,319	19,096	8,893	26
Rice					
Number of tests	49	50	49	50	48
Percent positive (%)	51	64	10	14	40
Average (ppb)	9	54	27	99	2
Median of positive (ppb)	5.0	66.2	310.0	148.0	3.5
1st quartile of positive (ppb)	3.0	45.0	114.0	140.0	2.0
3rd quartile of positive (ppb)	16.0	87.0	312.0	925.0	6.1
Maximum (ppb)	113	297	503	2,545	20
Finished feed					
Number of tests	1,164	1,354	1,407	1,173	850
Percent positive (%)	44	56	56	71	43
Average (ppb)	16	99	396	807	4
Median of positive (ppb)	13.0	60.0	374.0	727.0	2.5
1st quartile of positive (ppb)	4.0	36.0	212.3	339.5	1.0
3rd quartile of positive (ppb)	40.0	142.5	791.8	1,387.0	5.0
Maximum (ppb)	2,454	3,570	19,141	22,693	1,582
Straw					
Number of tests	24	24	24	24	24
Percent positive (%)	0	13	21	0	17
Average (ppb)	0	48	87	0	0
Median of positive (ppb)	-	418.0	400.0	-	2.0
1st quartile of positive (ppb)	-	326.5	299.0	-	1.2
3rd quartile of positive (ppb)	-	464.0	429.0	-	3.1
Maximum (ppb)	0	510	891	0	4
Barley					
Number of tests	24	47	45	23	15
Percent positive (%)	4	38	42	26	7
Average (ppb)	0	323	1,677	777	1
Median of positive (ppb)	1.8	72.7	1,210.0	1,574.5	9.6
1st quartile of positive (ppb)	1.8	45.8	675.5	1,109.3	9.6
3rd quartile of positive (ppb)	1.8	152.0	3,533.0	2,203.3	9.6
Maximum (ppb)	2	8,952	29,300	10,485	9.6

(Continued)

Table 7b. *Continues.*

Commodity/HPLC	Afla	ZON	DON	FUM	OTA
Other feed ingredients					
Number of tests	313	357	335	279	255
Percent positive (%)	27	27	29	12	29
Average (ppb)	25	76	183	190	3
Median of positive (ppb)	23.0	57.0	380.0	646.0	3.9
1st quartile of positive (ppb)	6.7	31.3	198.0	297.0	1.1
3rd quartile of positive (ppb)	63.0	138.5	662.0	1,438.0	12.5
Maximum (ppb)	570	6,587	10,320	11,333	111
Silage					
Number of tests	253	272	273	238	233
Percent positive (%)	2	29	38	19	14
Average (ppb)	0	81	333	167	1
Median of positive (ppb)	6.2	159.0	344.0	533.0	2.7
1st quartile of positive (ppb)	4.0	79.5	212.0	279.0	1.9
3rd quartile of positive (ppb)	8.0	421.5	657.0	895.0	6.5
Maximum (ppb)	9	2,146	14,326	3,134	35

U.S. Food and Drug Administration (FDA) guidelines for aflatoxins and deoxynivalenol's presence in cereals for use in animal feed and only 10% of the samples contained fumonisins levels higher than the recommendation for feeding equids and rabbits, an average of 500 ppb DON was reported for samples sourced from the Midwest of the United States and fumonisins level fluctuated between 100 to 7,200 ppb. An average of 600 ppb deoxynivalenol and 2,300 ppb fumonisins were found in phase II of the project, in samples sourced in Taiwan from different shipping containers originally from the U.S.. In comparison with maize and maize by-products, ingredients such as soybean and soybean meal, wheat and wheat bran, rice, rice bran and barley were in general, less contaminated. Moreover, aflatoxins are not a major contaminant of silages, but rather DON (38% positive), ZON (29% positive) and FUM (19% positive). This is in accordance with Scudamore and Livesey (1998) who reviewed previous work stating that the acidic conditions in silage are unfavorable for growth of *Aspergillus flavus* and therefore for the development of aflatoxins. In a study by Gonzalez Pereyra *et al.* (2007) the mean ZON value in fermented silage was reported to be 50 ppb, while for DON it was 276 ppb and for fumonisin B_1 it was 1,110 ppb. Other mycotoxins which could be of relevance in the case of this feedstuff, such as patulin, roquefortines A and C, mycophenolic acid and citrinin were not analyzed but their importance in roughage material must not be disregarded (Storm *et al.*, 2008). Regarding finished feed, almost all samples tested were positive for at least one mycotoxin (only 14% were negative for all tested mycotoxins, data not shown). FUM was present in 71% of tested samples (average 807 ppb) followed by DON (56%; average 396 ppb), ZON (56%; average 99 ppb), Afla (44%; average 16 ppb) and OTA (43%; average 4 ppb) (Gonzalez Pereyra *et al.*, 2007).

Results by mycotoxin

Figures 1A–E show the distribution of contamination levels found according to contamination range in ELISA-tested samples and Figures 2A–E show the distribution of contamination levels found according to contamination range in HPLC-tested samples. Once again the prevalence of mycotoxins in HPLC and ELISA analyzed samples

reflected the trends for the geographical regions from which the samples came, therefore HPLC-analyzed samples showed a higher prevalence of Afla, FUM and OTA. On the other hand, ELISA-tested samples were sourced mainly from Europe and North America (U.S. and Canada) where there is a higher prevalence of DON. Ranges were established for each mycotoxin separately due to their different toxicity levels. Thirty one % of HPLC-analyzed samples tested above 5 ppb Afla. Directive 2002/32/EC of the European Parliament and of the Council of 7 May 2002 sets 20 ppb as the maximum content of aflatoxin B_1 allowed for all feed materials intended for animal feed (Directive, 2002). Twelve % of HPLC-analyzed samples presented Afla levels above that threshold. Thirty % of samples which were analyzed for ZON had contamination levels above 50 ppb. The EU Recommendation of 17 August 2006 on the presence of deoxynivalenol, zearalenone, ochratoxin A, T-2 and HT-2 and fumonisins in products intended for animal feeding sets 8,000 ppb DON, 2,000 ppb ZON, 250 ppb OTA and 60,000 ppb FUM as guidance values for feed materials (cereals and cereal by-products with the exception of maize by-products) (Commission Recommendation, 2006). Twenty seven samples (0.7%) analyzed by HPLC had ZON levels above 2,000 ppb; 0.8% of samples (29 samples) had contamination levels above 8,000 ppb DON; no sample had FUM levels above 60,000 ppb; however 196 samples had contamination levels above 5,000 ppb FUM, the guidance level established by the Food and Agri-

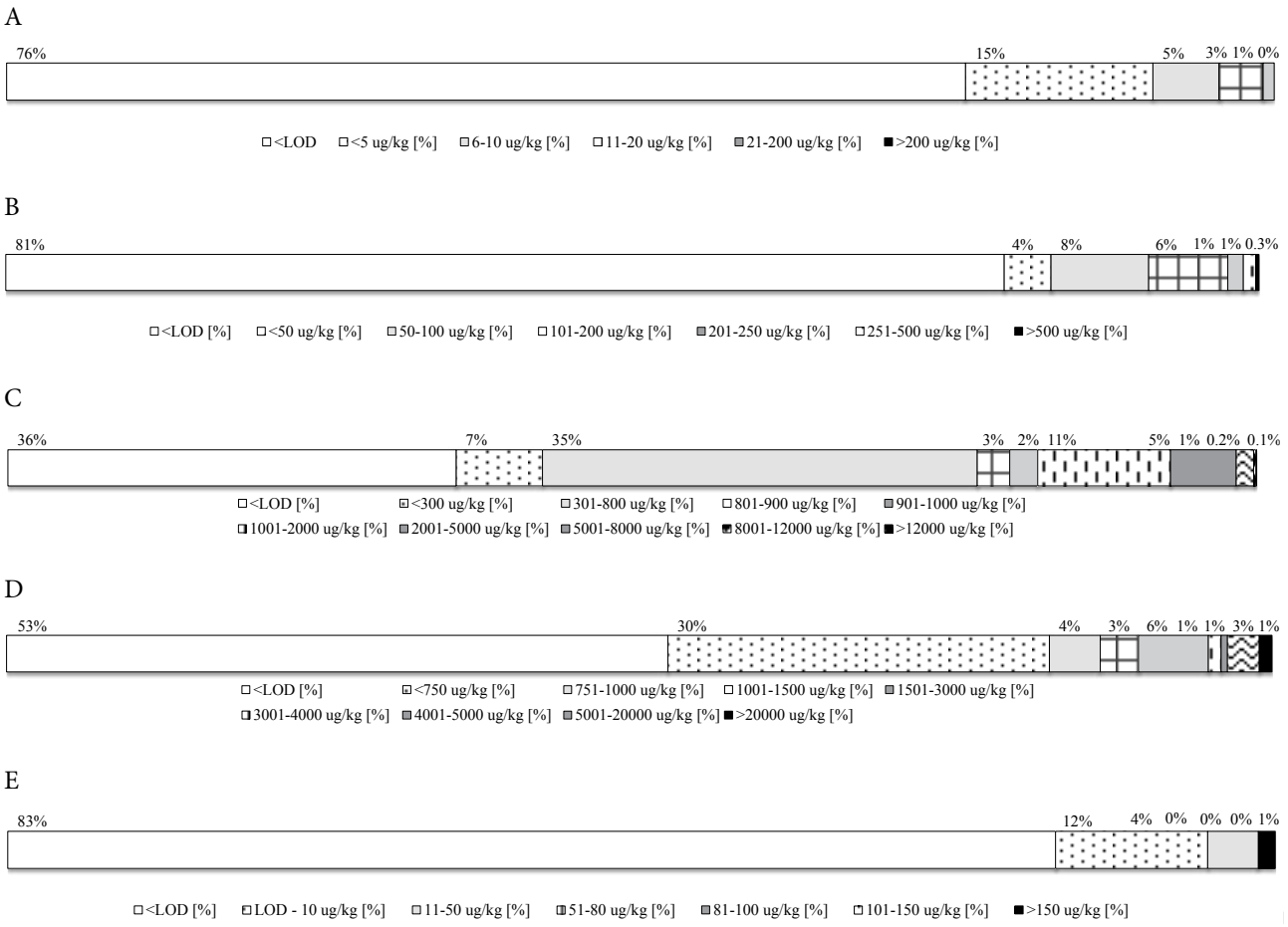

Figure 1. Distribution of mycotoxins occurrence by contamination range (percentage of samples), determined by ELISA. A, Afla; B, ZON; C, DON; D, FUM; E, OTA.

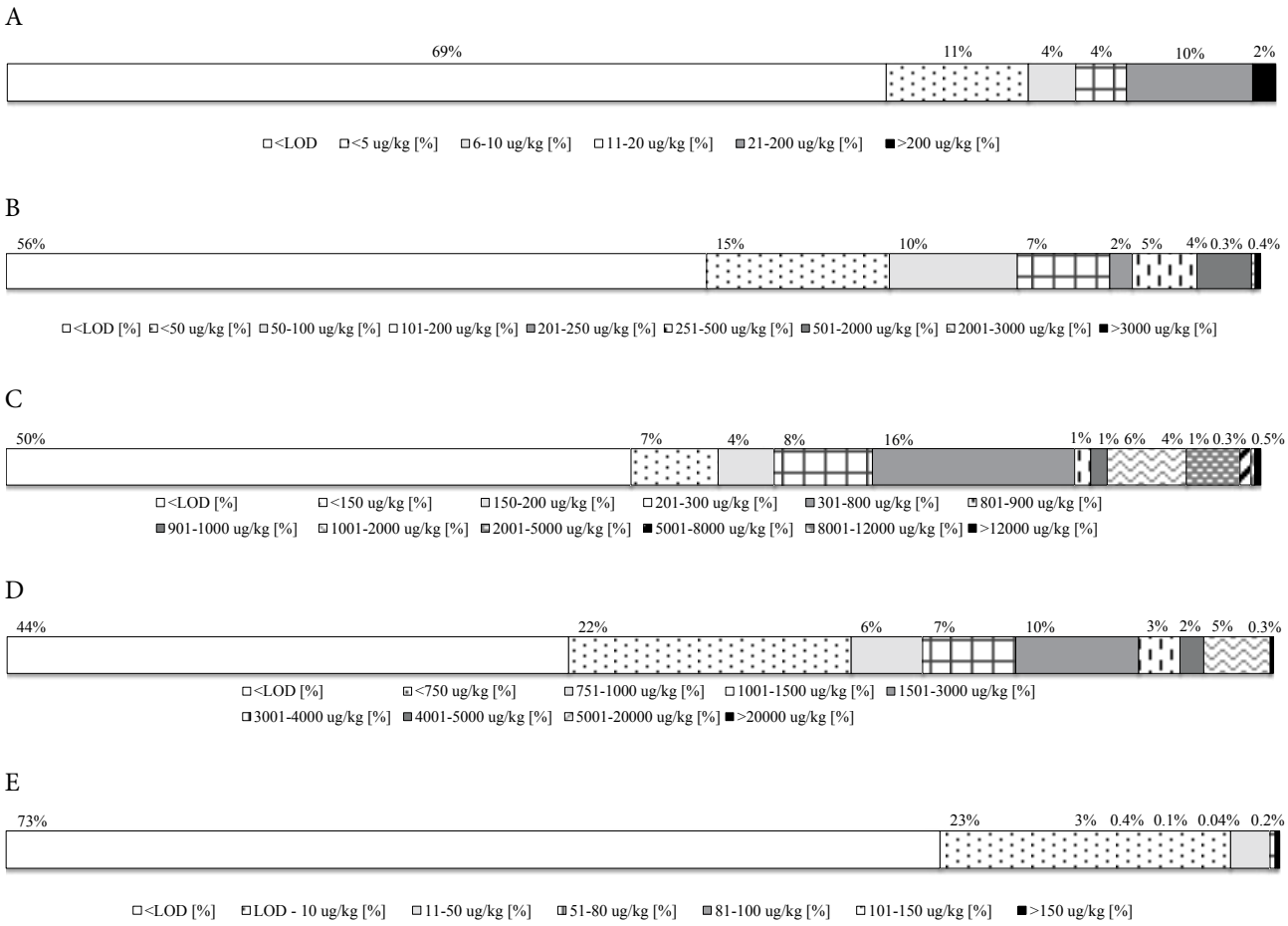

Figure 2. Distribution of mycotoxins occurrence by contamination range (percentage of samples), determined by HPLC. A, Afla; B, ZON; C, DON; D, FUM; E, OTA.

culture Organization (FDA) for feed ingredients used for equids and rabbits at no more than 20% of the diet (FDA, 2006). Six samples (0.3%) had OTA levels above 150 ppb, and a finished feed sample from Pakistan (1,582 ppb) had the maximum level found for this mycotoxin in the whole survey.

Figure 3 shows that within the samples tested by ELISA in the whole survey, 35% had mycotoxin levels below the LODs, 47% of the samples were contaminated by at least one mycotoxin and the remaining 18% had the presence of 2 or more mycotoxins. As for HPLC analysed samples, 19% had levels below the LODs, 31% had the presence of a single mycotoxin and 50% of them were contaminated with 2 or more mycotoxins. Multi-mycotoxin contamination is an area of great concern as it raises the risk of interactive effects classified as additive or synergistic effects.

Conclusions

This report shows the prevalence and concentration of mycotoxins in feedstuffs and feed used in the livestock production industry worldwide. A total of 20,417 mycotoxin analyzes were performed on 6,058 samples. Out of these, 65% of samples tested above ELISA limits of detection and 81% of samples analyzed by HPLC tested positive for at least one mycotoxin. Although in many cases the regulatory and guidance levels were not surpassed, attention should be given to the impact of the mycotoxins in animal health and performance.

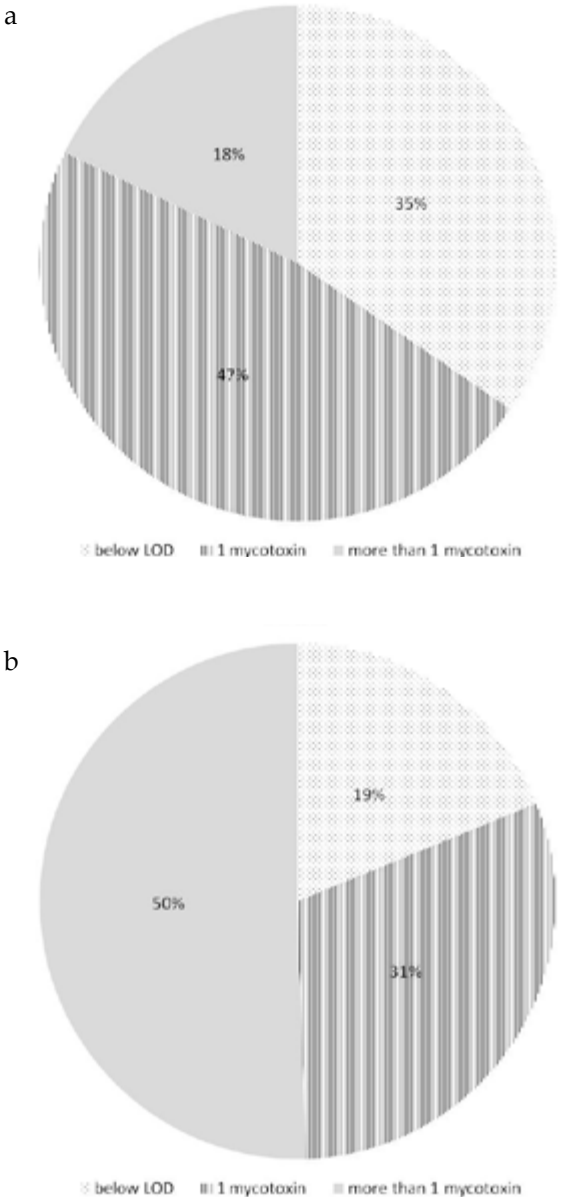

Figure 3. Co-occurrence of mycotoxins in analyzed materials (a, ELISA; b, HPLC).

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