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Mycotoxin co-contamination of foods and feeds: meta-analysis of publications describing toxicological interactions

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ABSTRACT

Most fungi are able to produce several mycotoxins simultaneously; moreover food and feed can be contaminated by several fungi species at the same time. Thus, humans and animals are generally not exposed to one mycotoxin but to several toxins at the same time. Most of the studies concerning the toxicological effect of mycotoxins have been carried out taking into account only one mycotoxin.

In the present review, we analyzed 112 reports where laboratory or farm animals were exposed to a combination of mycotoxins, and we determined for each parameter measured the type of interaction that was observed. Most of the published papers concern interactions with aflatoxins and other mycotoxins, especially fumonisins, ochratoxin A and trichothecenes. A few papers also investigated the interaction between ochratoxin A and citrinin, or between different toxins from *Fusarium* species. Only, experiments with a 2 x 2 factorial design with individual and combined effects of the mycotoxins were selected. Based on the raw published data, we classified the interactions in four different categories: synergistic, additive, less than additive or antagonistic effects.

This review highlights the complexity of mycotoxins interactions which varies according to the animal species, the dose of toxins, the length of exposure but also the parameters measured.

Keywords : co-contamination, toxicological synergism, animal health, feeding trials, chronic toxicity.

INTRODUCTION

Food safety is a major issue throughout the world. In this respect, much attention needs to be paid to the possible contamination of food and feed by fungi and the risk of mycotoxin production. Mycotoxins are secondary metabolites produced by fungi, mainly by species from the genus *Aspergillus*, *Fusarium* and *Penicillium*. Mycotoxins are very common contaminants of cereals. Furthermore, most mycotoxins are resistant to milling, processing and heating and, therefore, readily enter the food and feed chains (Bullerman and Bianchini, 2007). The

toxicological syndromes caused by ingestion of such toxins range from acute mortality, to slow growth and reduced reproductive efficiency. Consumption of fungal toxins may also result in impaired immunity and decreased resistance to infectious diseases (Oswald and Comera, 1998).

Most fungi are able to produce several mycotoxins simultaneously in separate feedstuffs, and considering, it is a common practice to use multiple grain sources in animals diets, the risk to be exposed to several mycotoxins at the same time increases. This is supported by global surveys that indicate humans and animals are generally exposed to more than one mycotoxin (Bermudez *et al.*, 1997; Boeira *et al.*, 2000; Monbaliu *et al.*, 2010; Rodrigues and Griessler, 2010; Speijers and Speijers, 2004; Wangikar *et al.*, 2004b). The toxicity of combinations of mycotoxins cannot always be predicted based upon their individual toxicities. Interactions between concomitantly occurring mycotoxins can be antagonistic, additive, or synergistic. The data on the *in vivo* combined toxic effects of mycotoxins are limited and therefore, the health risk from exposure to a combination of mycotoxins is incomplete.

In the present review, we only selected the *in vivo* experiments based on a 2 x 2 factorial design. Indeed such trials allow comparing the toxicological effects due to an exposure to a mycotoxins combination with the effects due to an exposure to single mycotoxins. Thus, we deliberately excluded the other multi-contamination experiments, especially the studies on grains naturally contaminated with *Fusarium* mycotoxins. Although of major interest in terms of frequency and relevance of contamination levels, the use of these contaminated materials does not allow to distinguish the type of interactions between each toxin present in the feed.

In spite of this restriction in our review, we summarized the published experiments, where laboratory and farm animals were exposed to a combination of mycotoxins, and described, parameter by parameter, the type of interaction observed.

CHARACTERIZATION OF THE DIFFERENT INTERACTIONS BETWEEN MYCOTOXINS

In the present review, in order to be consistent among the different papers, we went back to the original published raw data, and for each parameter that was measured, we classified the interaction in four different categories: synergistic, additive, less than additive and antagonistic effect.

- The “synergy” category was complex and we could differentiate 3 types of synergistic interaction (**Figure 1**) :

- ✓ Synergism type 1: contains experiments where the effect of the mycotoxins combination was greater than expected from the sum of the individual effects of the two toxins. We also included in this category, experiments where one toxin did not display any effect but where the effect of the co-contaminated treatment was greater than the effect of the other toxin alone (potentiation, P).
- ✓ Synergism type 2: the two mycotoxins induce opposite effects and the combined treatment induces an effect greater than the individual effect. So even though the toxins have opposite individual effects, the interaction led to an exacerbated effect.
- ✓ Synergism type 3: the two mycotoxins induce similar effects and the combined treatment induces an opposite effect than the individual effects.

- The “additive” category includes experiments where the effect of the combination could be calculated as the sum of the individual effects of the two toxins (**Figure 1**).

- The “less than additive” category includes experiments where the effect of the combined treatment mainly reflected the effect of only one of the toxin without additional effect of the other toxin (**Figure 1**).
- We also differentiated 2 types of antagonistic interaction (**Figure 1**) :
 - ✓ Antagonism type 1: the two mycotoxins induce similar effects and the combined treatment induces a lower effect. So, the combination lowered the effect of the more potent toxin.
 - ✓ Antagonism type 2: the two mycotoxins don’t induce the same effects and the combined treatment induces an effect intermediate between the two individual treatments. So, the combination lowered the effect of one of the toxin.

Results of the interaction were only reported, where significant differences were observed between the control group (no mycotoxin) and at least one of the mycotoxin-contaminated group (mycotoxin A, mycotoxin B or mycotoxin A+B), on parameters measured at the end of the experiment. Regarding histopathological analysis, few studies scored the incidence and severity of lesions; so we could not have analyzed the results based on the raw data, and include them in the different tables. Nevertheless, we included the conclusions as indicated by the authors in the text.

INTERACTION BETWEEN THE DIFFERENT MYCOTOXINS

Most of the published papers, dealing with the effect of mycotoxin multi-contamination on animals, concern aflatoxins (62/112 reports). The main mycotoxins investigated in association with aflatoxins are fumonisins (16 reports), ochratoxin A (19 reports), and trichothecenes (16 reports), especially T-2 toxin (11 reports). The other important interactions involved ochratoxin A and citrinin (11 reports), and, fumonisins and moniliformin (7 reports). Despite the high occurrence of mycotoxins from *Fusarium spp.*, only few studies have investigated the interaction between Fusariotoxins (23 reports).

I. INTERACTIONS BETWEEN AFLATOXINS AND OTHER MYCOTOXINS

1) Interaction between Aflatoxins (AF) and Fumonisins (FB)

a) effects of AF and FB on zootechnical parameters

As displayed in the **Table 1**, this association of mycotoxins resulted most of the time in a synergistic effect on body weight gain (Dilkin *et al.*, 2003; Harvey *et al.*, 1995b; Miazzo *et al.*, 2005; Ogido *et al.*, 2004; Orsi *et al.*, 2007; Pozzi *et al.*, 2001; Tessari *et al.*, 2006; Theumer *et al.*, 2008). Surprisingly, Pozzi *et al.* (2001) reported an increase and an antagonistic interaction on body weight gain when rats were fed both AF and FB.

Reduced egg production and egg weight is one of the effects of aflatoxicosis in laying hens. In quails, one study reported an antagonistic effect of AF and FB on egg production (**Table 1**). Surprisingly, egg production was reduced in animals fed FB-contaminated diet, and this effect was partially spared when animals received AF+FB contaminated diet (Ogido *et al.*, 2004).

b) effects AF and FB on biochemical parameters and organs weight

Several experiments have measured enzyme concentrations in the serum of animals fed multi-contaminated diets, in order to investigate tissue damage, especially the liver. The levels of mycotoxins used in these reports were moderate to high, and therefore induced organ

injuries, physiologically translated by increase of these biomarkers. The lack of response in some experiments is due to the low doses of mycotoxins used (Del Bianchi *et al.*, 2005). Overall, association of AF and FB induced a significant increase of the serum levels of AST, ALP, ALT and GGT (synergistic or additive effect, **Table 1**). Such increase in enzymatic activity can be attributed to cell necrosis, changes in cell membrane permeability or impairment of biliary excretion. This strong hepato-biliary dysfunction due to the ingestion of co-contaminated feed is in accordance with the histopathological observations (*see below*).

Another parameter correlated with hepatotoxicity, is the relative weight of liver (RW-L). Interestingly, either an increase or a decrease of the RW-L was observed in the experiments for the combined treatment (**Table 1**). This is attributed to the individual effect of each toxin, showing either an increase or a decrease for this parameter, according to studies. A synergistic interaction type 2 or 3 was recorded in pigs, turkeys, and rats exposed to both toxins (Harvey *et al.*, 1995b; Kubena *et al.*, 1995b; Pozzi *et al.*, 2001). It is possible that the mixture induced enough hepatic cell wall damage to have a major effect on total hepatic parenchymal mass. By contrast, other studies have reported an increase in the RW-L, but leading to a less than additive (Dilkin *et al.*, 2003; Tessari *et al.*, 2006) or an antagonistic interaction (Tessari *et al.*, 2006; Weibking *et al.*, 1994) in animals receiving multi-contaminated feed.

In two studies, ALP concentration was decreased when animals were fed with AF (Casado *et al.*, 2001; Kubena *et al.*, 1995b). The authors suggested that it was due to the inhibition of protein synthesis by AF. The combination of AF with FB led to a lesser reduction, suggesting an antagonistic interaction (**Table 1**).

c) effects of AF and FB on lipids

Disruption of sphingolipid biosynthesis is the main mechanism involved in FB toxicity, with inhibition of ceramide synthase leading to accumulation of sphingoid bases (sphinganine, Sa and sphingosine, So). This inhibition is well documented and the Sa/So ratio is considered as a good indicator of FB exposure (Theumer *et al.*, 2008).

Three studies analyzed the effect of co-exposure to AF and FB on sphingoid bases in different biological samples, with the aim of investigating if AF could exacerbate the toxicity of FB (Orsi *et al.*, 2007; Theumer *et al.*, 2008; Weibking *et al.*, 1994). These studies gave fairly different results (**Table 1**). In contrast with the two other studies, Orsi *et al.* (2007) observed an increased Sa/So ratio in urine of animals exposed to AF alone. Both, Orsi *et al.* (2007) and Weibking *et al.* (1994) demonstrated an antagonistic interaction between AF and FB on the Sa/So ratio in the liver and in the serum of exposed animals. Conversely, Theumer *et al.* (2008) observed a synergistic effect of the two toxins when looking at the Sa/So ratio in the kidney, urine, and to a lesser extent in the serum and the liver of exposed rats. Of note, this study also described a synergistic interaction in the incidence and the severity of kidney and liver lesions (Theumer *et al.* 2008).

d) effects of AF and FB on microscopic lesions

Microscopic lesions following ingestion of AF and FB were mostly evaluated in the liver and kidneys, their respective target organs. In these studies, additive or synergistic interactions between the two toxins were observed (Casado *et al.*, 2001; Del Bianchi *et al.*, 2005; Dilkin *et al.*, 2003; Gelderblom *et al.*, 2002; Harvey *et al.*, 1995b; Orsi *et al.*, 2007; Pozzi *et al.*, 2001; Tessari *et al.*, 2010; Tessari *et al.*, 2006; Theumer *et al.*, 2008; Weibking *et al.*, 1994). Histopathological alterations in liver consisted mainly in vacuolar degeneration of hepatocytes, apoptotic and mitotic figures, dysplastic nodules, megalocytosis and fibrosis. In animals receiving both AF and FB, cirrhotic livers were also observed. Enlargement of

gallbladder and duct proliferation were also reported (Orsi *et al.*, 2007). In kidney, apoptosis of tubular epithelial cells, glomerulonephritis and tubular epithelium with areas of degeneration and necrosis, as well as congestion and hemolysis were reported in animals fed AF and FB contaminated diet (Casado *et al.*, 2001; Del Bianchi *et al.*, 2005; Theumer *et al.*, 2008). These lesions were considered as moderate to severe, depending on the species and the doses used.

Lymphocytic infiltrates in the small intestine, and thickening of the alveolar walls and lymphocytic infiltrates, as well as apoptosis in the lungs, were observed in rats fed with diet containing AF (Theumer *et al.*, 2008). Incidence and severity of these alterations were similar in rats fed the co-contaminated diet, suggesting a less than additive interaction (**Table 1**).

e) effects of AF and FB on genotoxicity and carcinogenicity

Both AF and FB have carcinogenic effects, and besides, co-contamination of maize with AF and FB was related to a high-incidence area of human primary hepatocellular carcinoma in China (Li *et al.*, 2001). At the molecular level, the toxicology of AF involves its metabolic conversion by the cytochrome P450 system to the highly electrophilic AF-exo-8,9-epoxyde, which in turn binds to the DNA guanines to form adducts. Therefore, AF has been reported to be a good initiator and a proven complete carcinogen. On the other hand, FB has been described to be a potent tumor promoter but a weak initiator (Riley, 1998).

Two long-term trials, performed in rats and in rainbow trout, initiated hepatocarcinogenesis with AF, and then treated the animals with high doses of FB (Carlson *et al.*, 2001; Gelderblom *et al.*, 2002) (**Table 1**). In trout, FB promoted liver cancer in AF-initiated animals, but did not promote tumors in other tissues (Carlson *et al.*, 2001). Similarly, in rats, despite the fact that AF and FB were administered three weeks apart in a sequential model, they acted synergistically with respect to cancer initiation, as demonstrated by the number of hepatocytes nodules and foci (Gelderblom *et al.*, 2002).

Cellular oxidative stress was also proposed as a possible mechanism of cancer initiation. However, Theumer *et al.* (2010) only observed a less than additive and an antagonistic interaction between the two toxins when looking at DNA lesions using the alkaline comet assay (ACA) and the micronucleus technique (MN), respectively (**Table 1**). Furthermore, in the same study, a less than additive interaction was recorded in the MDA and CAT activity, both being biomarkers of oxidative stress.

f) effects AF and FB on immunity

Few studies investigated the combined effect of AF and FB on immunity (**Table 1**). After ingestion of the co-contaminated diet, the interaction on the reduced lymphocytes proliferation upon mitogenic stimulation, was described either as less than additive (Kubena *et al.*, 1995b) or additive (Harvey *et al.*, 1995b). Tessari *et al.* (2006) showed a synergistic decrease of the antibodies titers against Newcastle Disease. By contrast, Weibking *et al.* (1994) reported an unexpected increase and an additive effect of the two toxins when looking at the hemagglutination titers against SRBC in turkey poults.

Table 1 : Interaction between Aflatoxins (AF) and Fumonisin (FB)

Mycotoxins (Nature) Species (exposure)	Doses	SYNERGISTIC INTERACTION			ADDITIVE INTERACTION	LESS THAN ADDITIVE INTERACTION	ANTAGONISTIC INTERACTION		REF
		Type 1	Type 2	Type 3			Type 1	Type 2	
AF-FB (Pu – Pu) Fish (322 d) ¹	0.1 – 3					- tumors in AF-initiated liver ↗			Carlson <i>et al.</i> , 2001
AF-FB (Ex – Pu) Rat (21 d)	0.72 bw/d – 5 bw/d				- RW-L ↗ - AST, ALT ↗		- BWG ↗ - RW-K ↓ - ALP, GGT ↗ - TP, creatinine, albumin ↗	- urea →	Pozzi <i>et al.</i> , 2001
	0.72 bw/d – 15 bw/d	- GGT ↗	- BWG ↓ - RW-L ↓		- AST, ALP, ALT ↗ - TP, albumin ↗	- RW-K ↓	- creatinine ↗	- urea ↗	
AF-FB (Ex – CM) Chicken (33 d)	0.05 – 50	- AST ↗							Tessari <i>et al.</i> , 2010
	0.05 – 200				- AST ↗				
	0.2 – 50						- AST ↗		
	0.2 – 200				- TP ↗		- AST ↗		
AF-FB (Ex – CM) Chicken (33 d)	0.05 – 50	- BWG ↓ - Ab ND ↓ (P)			- RW-H, BF ↗				Tessari <i>et al.</i> , 2006
	0.05 – 200	- Ab ND ↓			- BWG ↓ - RW-H ↗		- RW-L ↗ - RW-BF →		
	0.2 – 50	- RW-BF ↗ - Ab ND ↓			- BWG ↓	- RW-L ↗	- RW-H ↗		
	0.2 – 200	- Ab ND ↓ (P)			- BWG ↓ - RW-H ↗	- RW-BF ↗	- RW-L ↗		
AF-FB (Pu – Ex) Rat (90 d)	0.04 – 100	- SOD activity ↗				- DNA damage in SMC (ACA) ↗ - MDA, CAT activity ↗	- DNA damage in SMC (MN) ↗		Theumer <i>et al.</i> , 2010
AF-FB (Pu – Ex) Rat (90 d)	0.04 – 100	- BW ↓ (P) - Incidence, severity of liver & kidney lesions ↗ - Sa/So kidney, urine, serum, liver ↗ (P)				- feed intake ↓ - Incidence, severity of lung & intestine lesions ↗			Theumer <i>et al.</i> , 2008
AF-FB (Pu – CM) Turkey (21 d)	0.2 – 75	- RW-Pc ↗			- Ab SRBC ↗	- BWG ↓ - RW-S ↗ - albumin, TP, cholesterol ↓	- AST ↗ - hemoglobin ↗	- RW-BF → - RW-L ↗ - Sa/So serum ↗	Weibking <i>et al.</i> , 1994

1) Carlson *et al.* (2001) : 30 min initiation by immersion with AF, followed by 4 weeks of recovery, and then, 42 weeks FB feeding 2) Gelderlom *et al.* (2002) : 14 days initiation with AF, followed by 3 weeks of recovery, and then 3 weeks FB feeding

- **Nature of mycotoxins** : Pu, Purified (>90%); Ex, Extracted from fungal culture material (partially purified); CM, Culture Material; NCF, Naturally Contaminated Feedstuffs; NI, No Indicated in the study

- λ , \sphericalangle , \rightarrow : evaluation at the end of each experiment, of the effect of the co-contaminated treatment in comparison to the control treatment.

- (P), potentiation

- unless stated, doses are expressed in mg of toxins/kg of feed. In few experiments, doses are expressed in mg of toxins/kg of body weight/day and in mg of toxins/day, and indicated as bw/d and mg/d, respectively.

- **zootechnical parameters** : BW(G), body weight (gain)

- **RW, relative weight of organs** : RW-L, liver; -K, kidney; -G, gizzard; -Pv, proventriculus; -Pc, pancreas; -H, heart; -Lg, lung; -A, adrenals; -S, spleen; -T, thymus; -BF, bursa of Fabricius.

- **biochemical and hematological parameters** : TP, total proteins; BUN, blood urea nitrogen; RBC, red blood cells; WBC, white blood cells; Sa, sphinganine; So, sphingosine; MDA, malondialdehyde; PC, protein carbonyl; CDNB, 1-chloro-2,4-dinitrobenzene; DCNB, 1,2-dichloro-4-nitrobenzene.

- **enzymatic parameters** : ALT, alanine aminotransferase; ALP, alkaline phosphatase; AST, aspartate aminotransferase; GGT, gamma-glutamyltransferase; LDH, lactate dehydrogenase; GDH, glutamate dehydrogenase; CK, creatine kinase; CHL, cholinesterase; CAT, catalase; SOD, superoxide dismutase; GSTP⁺, glutathione-S-transferase placental form positive; EROD, ethoxyresorufin *O*-dealkylase; PROD, pentoxyresorufin *O*-dephthylase

- **immune parameters** : SMC, spleen mononuclear cells; Ab, antibodies; ND, Newcastle disease; IDB, infectious bursal disease; SRBC, sheep red blood cells; DTH, delayed-type hypersensitivity.

2) Interaction between Aflatoxins (AF) and Ochratoxin A (OTA)

a) effects of AF and OTA on zootechnical parameters

As indicated in **Table 2**, the interaction between AF and OTA on animal performance has been studied in 11 publications. In most of these studies, the association induced a synergistic or an additive effect on body weight gain (Harvey *et al.*, 1989a; Huff and Doerr, 1981; Huff *et al.*, 1983; Huff *et al.*, 1992; Sakhare *et al.*, 2007; Verma *et al.*, 2004). In addition, Huff *et al.* (1983) observed that even four weeks after exposure to the co-contaminated diet had stopped, the animals did not recover their normal body weight. The same author also observed that in chickens, ingestion of feed co-contaminated with AF and OTA decreased carcass yield, breast weight and other processing parameters in a synergistic manner (Huff *et al.*, 1984) (**Table 2**).

Both AF and OTA affect egg production and hatchability, and one group investigated the combined effect of these two toxins on laying hens (Verma *et al.*, 2003; 2007) (**Table 2**). An additive interaction of AF and OTA was observed on egg production and on the feed efficiency (consumption for egg production) (Verma *et al.*, 2003). Regarding egg quality, less than additive and antagonistic interactions were observed on eggs. Protein and energy utilization were also modulated by the consumption of AF and OTA. However the interaction between the two toxins varied according to their concentrations ranging from synergistic to less than additive effects (Verma *et al.*, 2007).

b) effects AF and OTA on biochemical parameters and organs weight

Regarding biochemical parameters, many studies reported a less than additive or an antagonistic interaction between AF and OTA (**Table 2**). These types of response were observed for serum concentrations of cholesterol, albumin, total proteins, creatinine, uric acid or blood urea nitrogen (Harvey *et al.*, 1989a; Huff *et al.*, 1992; Kalorey *et al.*, 2005; Raju and Devegowda, 2000; Richard *et al.*, 1975; Sakhare *et al.*, 2007; Tapia and Seawright, 1985).

Several studies have assessed the effect of the co-contaminated diet on relative weight of liver and on liver functions (**Table 2**). The type of interaction between the two toxins on the increased relative weight of the liver varied a lot from one experiment to another. Huff and Doerr (1981), and Harvey *et al.* (1989) observed a synergistic interaction, while Huff *et al.* (1992) and Verma *et al.* (2004) observed an additive interaction. Kalorey *et al.* (2005) noted a less than additive interaction, and with higher doses of toxins, Verma *et al.* (2004) described an antagonistic interaction. Antagonism was also observed in the experiments of Raju and Devegowda (2000) and Sakhare *et al.* (2007). Interestingly, OTA seems to inhibit or spare the effect of AF on the accumulation of lipid in the liver, leading to an antagonistic interaction between the two mycotoxins (Huff and Doerr, 1981; Huff *et al.*, 1984) (**Table 2**). Few data are available on the biomarkers of hepatotoxicity, but an additive or less than additive effect was observed for the increased concentrations of ALP (Harvey *et al.*, 1989a; Kalorey *et al.*, 2005) and GGT (Raju and Devegowda, 2000), and an antagonistic or less than additive effect was reported for the decreased concentrations of AST (Huff *et al.*, 1992) and ALT (Raju and Devegowda, 2000) (**Table 2**).

Concerning the increased relative weight of kidney, as already mentioned for the liver, the type of interaction of the two toxins on this organ range from synergism to antagonism (Harvey *et al.*, 1989a; Huff and Doerr, 1981; Huff *et al.*, 1992; Raju and Devegowda, 2000; Verma *et al.*, 2004) (**Table 2**).

Based on the relative weights of gizzard, proventriculus and pancreas, it seems that the upper alimentary tract is more sensitive to the combination than the mycotoxins alone (Huff and Doerr, 1981; Huff *et al.*, 1992) (**Table 2**).

Of note, a lack of response for most of the variables studied was observed in calves fed contaminated diets (Patterson *et al.*, 1981). This failure was probably attributable to the low doses of mycotoxins used, and to the detoxifying action of the rumen flora on OTA.

c) effects of AF and OTA on microscopic lesions

Microscopic lesions following ingestion of AF and OTA contaminated feed were mostly evaluated in the liver and kidneys, their respective target organs (Huff and Doerr, 1981). Unfortunately, the different studies are not in agreement with each other. For example, in chickens, OTA in the diets prevented hepatic fatty infiltration that was caused by AF (Huff and Doerr, 1981). Pigs fed the co-contaminated diet, presented the same hepatic lesions as those fed diet contaminated with AF alone (Tapia and Seawright, 1985). By contrast, Sakhare *et al.* (2007) recorded more severe hepatic lesions in chickens receiving the co-contaminated diet, with granular and vacuolar degenerative changes, necrosis of liver parenchyma and areas of hemorrhages.

The same discrepancy was noticed for the histology of the kidney. In pigs, Tapia and Seawright (1985) observed less severe renal lesions in animals fed the co-contaminated diet than in animals receiving the OTA-contaminated diet. This is in agreement with the data obtained in pigs, by Harvey *et al.* (1989a) on the relative weight of kidney, and the data obtained by Tapia and Seawright (1985) on the creatinine and blood urea nitrogen concentrations (**Table 2**). Conversely, Sakhare *et al.* (2007) indicated that renal injuries appeared earlier and were more developed in chickens fed a multi-contaminated diet than in animals receiving the mono-contaminated diets. This led to destruction of tubular epithelium, with detachment of tubular cells from basement membrane. The species used may explain these discrepancies.

One study focused on the effect of AF and OTA on bruising in broiler chickens (Huff *et al.*, 1983), as bloody thigh syndrome has been suspected to be associated with mycotoxicoses. This study revealed a severe coagulopathy, as measured by elevated prothrombin times, and, an additive and an antagonistic interaction between AF and OTA were observed for the incidence and severity of breast and right thigh bruises, respectively (**Table 2**).

d) effects of AF and OTA on immunity

Atrophy of lymphoid organs were observed in several studies (Kalorey *et al.*, 2005; Raju and Devegowda, 2002; Sakhare *et al.*, 2007; Verma *et al.*, 2004) (**Table 2**). This seems to be due to necrosis and cellular depletion, as suggested by microscopical observations with necrotic areas in germinal centre, depopulation of lymphocytes in spleen, or depletion and necrosis of lymphoid cells from follicle in bursa of Fabricius (Sakhare *et al.* 2007). In the bursa of Fabricius, these histopathological changes were more pronounced in animals fed the co-contaminated diet compared to animal receiving the mono-contaminated diets (Sakhare *et al.*, 2007).

The depletion of lymphocytes suggested a suppression of cell mediated immunity. Indeed, the contact sensitivity reaction, was reduced in chickens fed the co-contaminated diet with an additive interaction between AF and OTA (Kalorey *et al.*, 2005; Sakhare *et al.*, 2007; Verma *et al.*, 2004). However, Verma *et al.* (2004) observed that, with increasing doses of toxins, the interaction went from additive to less than additive to antagonistic (**Table 2**).

In order to analyze the effect of the mycotoxins on the humoral response, the antibodies titers against Newcastle disease, Sheep red blood cells or Infectious Bursal disease were measured (Kalorey *et al.*, 2005; Raju and Devegowda, 2002; Sakhare *et al.*, 2007; Verma *et al.*, 2004). Mono- and multi-contaminated diets decreased the humoral response, and interaction between AF and OTA showed either an additive (Sakhare *et al.*, 2007; Verma *et*

al., 2004) or antagonistic interaction (Kalorey *et al.*, 2005; Raju and Devegowda, 2002; Verma *et al.*, 2004) (**Table 2**).

Richard *et al.* (1975) also investigated the effect of OTA and AF on complement activity and reported a less than additive interaction between the two toxins (**Table 2**).

e) effects of AF and OTA on teratology

Both AF and OTA are able to cross the placental barrier and demonstrated some teratogenic properties in some species (Wangikar *et al.*, 2004b; Wangikar *et al.*, 2005)

In two separate experiments, Edrington *et al.* (1995) showed that embryonic mortality increased following injection of a combination of AF and OTA, but led to different types of interaction according to doses and experiments. He suggested these differences were related to variations in embryo sensitivities among batches of eggs. By contrast, exposure of chicken embryos to the combination of toxins did mostly result in a synergistic interaction in the number of abnormalities.

Wangikar in 2004b and 2005 performed two large studies in rats and rabbits, respectively, where mycotoxins were administered orally on days 6-18 of gestation. Both mycotoxins interfered with the body wall formation leading to gastroschisis as observed in both the high OTA and high AF combination groups (Wangikar *et al.*, 2004b). In the co-exposed group, more severe cardiac lesions were noticed (Wangikar *et al.*, 2004b; Wangikar *et al.*, 2005) but skeletal, and visceral anomalies were reduced or absent, when compared to the mono-exposed groups. Similarly, the presence of AF seems to prevent the exencephaly and incomplete closure of the skull caused by OTA; and the presence of OTA prevent the head abnormality and open eye caused by AF. Another study of the same group also indicates that brain, kidney, and liver lesions were less severe in rats fetuses co-exposed to AF and OTA than in mono-exposed animals (Wangikar *et al.*, 2004a). Two mechanisms were proposed to explain these observations. The first hypothesis is that AF may inhibit the metabolism of OTA in the liver and increase its excretion. The second hypothesis is based on the property of OTA and AF to inhibit protein synthesis. Indeed, OTA is known to limit protein synthesis through competitive inhibition of phenylalanine-t-RNA-synthesis with phenylalanine, while AF prevented protein synthesis through an inhibition of RNA synthesis. Therefore, antagonistic effects of the mycotoxins might be due to inhibition of transcription by AF, with a concomitant increase in the cellular pool of phenylalanine available for translation, as inhibition of protein synthesis by OTA was inversely proportional to phenylalanine concentration (Wangikar *et al.*, 2004b).

Table 2 : Interaction between Aflatoxins (AF) and Ochratoxin A (OTA)

Mycotoxins (Nature) Species (exposure)	Doses	SYNERGISTIC INTERACTION			ADDITIVE INTERACTION	LESS THAN ADDITIVE INTERACTION	ANTAGONISTIC INTERACTION		REF
		Type 1	Type 2	Type 3			Type 1	Type 2	
AF-OTA (Pu – Pu) Chicken egg (10 d) ¹	0.025 – 1.15 (µg/egg)					- embryos mortality ↗		- abnormalities ↗	Edrington <i>et al.</i> , 1995
	0.05 – 2.3 (µg/egg)	- abnormalities ↗					- embryos mortality ↗		
AF-OTA (Pu – Pu) Chicken egg (15 d) ¹	0.025 – 1.0 (µg/egg)	- abnormalities ↗			- embryos mortality ↗				Edrington <i>et al.</i> , 1995
	0.05 – 2.0 (µg/egg)	- abnormalities ↗			- embryos mortality ↗				
AF-OTA (CM – Pu) Pig (28 d)	2.0 – 2.0	- triglycerides ↗ (P)	- TP ↗ - RW-L ↗		- BW ↓ - ALP ↗ - inorganic phosphorus ↓	- BUN, cholesterol, glucose ↓	- calcium → - sodium ↓	- albumin → - hemoglobin → - RW-K →	Harvey <i>et al.</i> , 1989a
AF-OTA (CM – Pu) Chicken (21 d)	2.5 – 2.0	- BW ↓ - mortality ↗ - RW-L,K,G,S,Pv,Pc ↗						- liver lipid levels →	Huff and Doerr, 1981
AF-OTA (CM – Pu) Chicken (42 d)	2.5 – 2.0	- BW ↓ - prothrombin times ↗			- incidence and severity of breast bruises ↗		- incidence and severity of right thigh bruises ↗		Huff <i>et al.</i> , 1983
AF-OTA (CM – Pu) Chicken (42 d)	2.5 – 2.0	- live, dressed, eviscerated weight ↓ - carcasse yield, breast, drum, thigh, wing, back weights ↓ - plasma carotenoids ↓				- breast yield ↓		- liver lipid levels ↗	Huff <i>et al.</i> , 1984
AF-OTA (CM – Pu) Chicken (21 d)	3.5 – 2.0				- BW ↓ - RW-L,K,H,Pv ↗		- albumin, TP, cholesterol ↓	- uric acid ↗ - AST ↓	Huff <i>et al.</i> , 1992
AF-OTA (CM – CM) Chicken	0.2 – 0.2				- RW-S ↓ - DTH reaction ↓	- hemoglobin ↓ - albumin, cholesterol, triglycerides ↓	- Ab ND ↓		Kalorey <i>et al.</i> , 2005

(42 d)						- creatinine, uric acid ↗ - ALP ↗ - RW-L ↗ - RW- BF,T ↘			
AF-OTA (NCF–NCF) <i>Calve</i> (87 d)	0.013 – 0.450						- WBC ↘		Patterson <i>et al.</i> , 1981
AF-OTA (CM – CM) <i>Chicken</i> (35 d)	0.3 – 2.0				- RW-G,A ↗ - GGT ↗ - hemoglobin ↘	- BW, feed intake ↘ - ALT ↘	- mortality ↗ - RW-L,K ↗ - TP, cholesterol, BUN ↘ -coagulation time →		Raju and Devegowda, 2000
AF-OTA (CM – CM) <i>Chicken</i> (35 d)	0.3 – 2.0						- RW-BF,T ↘ - Ab ND, IBD ↘		Raju and Devegowda, 2002
AF-OTA (Ex – Pu) <i>Guinea pig</i> (28 d)	0.01 mg/d – 0.45 mg/d					- TP, globulin ↘ - complement titer ↘			Richard <i>et al.</i> , 1975
AF-OTA (CM – CM) <i>Chicken</i> (42 d)	0.2 – 0.2				- BW ↘ - cholesterol ↘ - RW-S,T ↘ - Ab ND, DTH reaction ↘	- triglyceride ↘ - creatinine, uric acid ↗	- hemoglobin ↘ - RW-L ↗, RW-BF ↘	- WBC →	Sakhare <i>et al.</i> , 2007
AF-OTA (Ex – Pu) <i>Pig</i> (42 d)	0.375 – 1.0					- BWG ↘	- creatinine ↗	- BUN →	Tapia and Seawright, 1985
AF-OTA (NI – NI) <i>Laying hen</i> (50 d)	0.5 – 1.0	- feed consumed for production of 1 dozen eggs ↗			- egg production ↘	- egg shape index ↘	- feed intake ↘ - egg shell thickness →		Verma <i>et al.</i> , 2003
	1.0 – 2.0				- egg production ↘ - feed consumed for production of 1 dozen eggs ↗ - egg shape index ↘		- feed intake ↘	- egg shell thickness ↘	
	2.0 – 4.0				- egg production ↘ - feed consumed for production of 1 dozen eggs ↗	- feed intake ↘ - egg shape index ↘	- egg shell thickness →		

AF-OTA (CM – CM) Laying hen (50-75 d)	0.5 – 1.0	- metabolizable energy, protein retention √ - maintenance energy requirement ↗			- egg energy deposition √				Verma <i>et al.</i> , 2007
	1.0 – 2.0	- maintenance energy requirement ↗			- metabolizable energy, protein retention, egg energy deposition √				
	2.0 – 4.0				- metabolizable energy, protein retention √	- egg energy deposition √ - maintenance energy requirement ↗			
AF-OTA (CM – CM) Chicken (49 d)	0.5 – 1.0	- BWG √ - feed intake √			- RW-L ↗ - DTH reaction √		- Ab SRBC √		Verma <i>et al.</i> , 2004
	1.0 – 2.0	- feed intake √			- BWG √ - RW-L ↗ - Ab SRBC √	- RW-BF √ - DTH reaction √		- RW-K ↗	
	2.0 – 4.0		- RW-K ↗		- BWG √ - feed intake √ - Ab SRBC √	- RW-BF √	- RW-L ↗ - DTH reaction √		

1) Edrington *et al.* (1995) : one administration and measurements 10 or 15 d post-administration

- **Nature of mycotoxins** : Pu, Purified (>90%); Ex, Extracted from fungal culture material (partially purified); CM, Culture Material; NCF, Naturally Contaminated Feedstuffs; NI, No Indicated in the study
- ↗, √, → : evaluation at the end of each experiment, of the effect of the co-contaminated treatment in comparison to the control treatment.
- (P), potentiation
- unless stated, doses are expressed in mg of toxins/kg of feed. In few experiments, doses are expressed in mg of toxins/kg of body weight/day and in mg of toxins/day, and indicated as bw/d and mg/d, respectively.
- **zootechnical parameters** : BW(G), body weight (gain)
- **RW, relative weight of organs** : RW-L, liver; -K, kidney; -G, gizzard; -Pv, proventriculus; -Pc, pancreas; -H, heart; -Lg, lung; -A, adrenals; -S, spleen; -T, thymus; -BF, bursa of Fabricius.
- **biochemical and hematological parameters** : TP, total proteins; BUN, blood urea nitrogen; RBC, red blood cells; WBC, white blood cells; Sa, sphinganine; So, sphingosine; MDA, malondialdehyde; PC, protein carbonyl; CDNB, 1-chloro-2,4-dinitrobenzene; DCNB, 1,2-dichloro-4-nitrobenzene.
- **enzymatic parameters** : ALT, alanine aminotransferase; ALP, alkaline phosphatase; AST, aspartate aminotransferase; GGT, gamma-glutamyltransferase; LDH, lactate dehydrogenase; GDH, glutamate dehydrogenase; CK, creatine kinase; CHL, cholinesterase; CAT, catalase; SOD, superoxide dismutase; GSTP⁺, glutathione-S-transferase placental form positive; EROD, ethoxyresorufin O-dealkylase; PROD, pentoxyresorufin O-depentylase
- **immune parameters** : SMC, spleen mononuclear cells; Ab, antibodies; ND, Newcastle disease; IDB, infectious bursal disease; SRBC, sheep red blood cells; DTH, delayed-type hypersensitivity.

3) Interaction between Aflatoxins (AF) and Trichothecenes (TCT)

3.1) Interaction between Aflatoxins (AF) and T-2 toxin (T2)

a) effects of AF and T-2 toxin on zootechnical parameters

Several studies have investigated the combined effect of AF and T-2 toxin on body weight gain (**Table 3**), and all of them demonstrated at least an additive, or a synergistic interaction between the two toxins (Girish and Devegowda, 2006; Harvey *et al.*, 1990; Huff *et al.*, 1988; Kubena *et al.*, 1990; Madheswaran *et al.*, 2005; Raju and Devegowda, 2000). A similar interaction was also observed when looking at the relative weight of several organs of poultry (Girish and Devegowda, 2006; Huff *et al.*, 1988; Kubena *et al.*, 1990; Raju and Devegowda, 2000; 2002). By contrast, in pigs and in rats, Harvey *et al.* (1990) and Tamimi *et al.* (1997) observed an antagonistic interaction on the relative weights of liver, heart and kidney (**Table 3**).

b) effects of AF and T-2 toxin on biochemical parameters

Regarding biochemical parameters, the combined treatment showed a significant decrease for several parameters, such as total proteins, albumin, cholesterol, triglycerides, as well as some enzyme concentrations (ALP, ALT, LDH) (**Table 3**). These reduced concentrations may be attributed to the property of both AF and T-2 toxin to inhibit protein synthesis, during transcription and translation respectively. Nonetheless, among these studies, different types of interaction were observed for these biochemical parameters, ranging from synergistic to antagonistic interactions (**Table 3**).

c) effects of AF and T-2 toxin on microscopic lesions

Because T-2 toxin is a potent irritant of the buccal cavity, the effect of AF and T-2 toxin was investigated on the oral cavity. When chickens received mono-contaminated feed, oral lesions were only seen in birds given T-2 toxin, and the combination with AF resulted in an antagonistic interaction (Kubena *et al.*, 1990) (**Table 3**). The authors suggested that the lower effect of T-2 toxin in the presence of AF was likely due to a decreased intake of T-2 toxin in chicks that received the multi-contaminated diet. In swine, Harvey *et al.* (1990) reported necrotizing contact dermatitis on the snout, buccal commissures, and prepuce of animals. However, in the publication, the authors indicated that these dermal lesions were observed in T-2 and T-2+AF groups, but did not mention the type of interaction.

The interaction of AF and T-2 toxin led to contradictory results concerning the lesions observed in the liver. Indeed, a synergistic effect was observed in rats with hepatic injuries characterized by congestions with fatty acid changes, and significant bile duct proliferation (Tamimi *et al.*, 1997). An additive effect was observed in quails characterized by fatty vacuoles, mitochondria degeneration, and indistinguishable rough endoplasmic reticulum (Madheswaran *et al.*, 2006). By contrast, less than additive and antagonistic effects were reported in chickens and swine fed multi-contaminated diets respectively, in comparison to the lesions observed in animals exposed to AF-contaminated diet (Harvey *et al.*, 1990; Kubena *et al.*, 1990). These data correlate with the interactions observed on biochemical parameters for the two toxins.

A synergistic effect of the two toxins was also observed for renal lesions, with tubular epithelial degeneration, congestion, swelling of the glomureli, as well as hypercellularity (Tamimi *et al.*, 1997).

d) effect on immunity

One research group has investigated the interaction between AF and T-2 toxin on the immune system (Girish and Devegowda, 2006; Raju and Devegowda, 2002). Conclusions between these two studies were in agreement and showed different types of interaction of the two mycotoxins depending on the parameter : a synergistic or additive effect on the thymus weight, an additive effect on the bursa of Fabricius weight, a less than additive effect on the antibody titer against bursal disease infection, and an antagonistic effect on the antibody titer against Newcastle disease (**Table 3**).

3.2) Interaction between Aflatoxins (AF) and Diacetoxyscirpenol (DAS)

The interaction between AF and diacetoxyscirpenol (DAS) was reported in three studies performed in lambs, pigs and chickens (Harvey *et al.*, 1995a; Harvey *et al.*, 1991; Kubena *et al.*, 1993). The levels of mycotoxins used in these studies were quite similar and the association of toxins led to a synergistic or additive interaction on body weight gain (**Table 3**).

Like T-2 toxin, DAS has been described as radiomimetic with regard to lymphoid tissues and gastrointestinal epithelium, as a contact necrotizing agent for lingual and buccosal mucosa. However, oral lesions were only observed in chickens, the most susceptible species (Kubena *et al.* 1993).

The association of AF and DAS led to a less than additive or an antagonistic interaction on most biochemical parameters investigated, and also with either increased or decreased values for the same compounds (cholesterol, TP, enzyme concentrations) depending on species (**Table 3**).

In liver, lesions reported (vacuolar changes accompanied by early portal fibrosis) were similar in nature and severity between animals exposed to AF and AF-DAS treatments (Harvey *et al.*, 1995a; Harvey *et al.*, 1991).

3.3) Interaction between Aflatoxins (AF) and Deoxynivalenol (DON)

Two experiments were conducted in pigs and chickens to study the interaction between AF and DON (Harvey *et al.*, 1989b; Huff *et al.*, 1986). In both experiments, body weight gain was decreased with an additive or less than additive interaction between the two toxins (**Table 3**). The main difference between the two experiments was the individual effect of DON. Harvey *et al.* (1989b) only observed minor effects of DON, and for most parameters he measured, the interaction with AF was less than additive or antagonistic. Conversely, Huff *et al.* (1986), who used high concentration of DON, reported an important effect of the toxin. He also observed less than additive or antagonistic interaction between DON and AF for the different parameters he measured (**Table 3**).

At the histological level, mono-contaminated feed with either DON or AF induced edema of the gastric mucosa. By contrast, animals fed the multi-contaminated diet did not show any gastric lesions (Harvey *et al.*, 1989b). In liver, mild hepatic interlobular fibrosis, bile duct hyperplasia, and diffuse hepatocellular lipidosis were only observed in the groups fed AF, either mono- or multi-contaminated groups, and suggested less than additive interaction (Harvey *et al.*, 1989b).

Table 3 : Interaction between Aflatoxins (AF) and Trichothecenes (TCT)

Mycotoxins (Nature) Species (exposure)	Doses	SYNERGISTIC INTERACTION			ADDITIVE INTERACTION	LESS THAN ADDITIVE INTERACTION	ANTAGONISTIC INTERACTION		REF
		Type 1	Type 2	Type 3			Type 1	Type 2	
AF-T2 (CM – CM) Chicken (35 d)	2.0 – 1.0	- RW-K ↗ (P)			- BWG ↘ - RW-L ↗ - RW-T,BF ↘	- feed intake ↘ - RW-G,S ↗ - Ab IBD ↘	- Ab ND ↘		Girish and Devegowda, 2006
AF-T2 (CM – Pu) Pig (28 d)	2.5 – 10				- BWG ↘	- BUN ↘ - GGT ↗	- sodium, phosphorus, albumin ↘	- cholesterol, triglycerides, TP ↘ - ALP, CHL ↗, AST → - RBC, WBC ↗, hemoglobin → - RW-L ↗, RW-H,K →	Harvey <i>et al.</i> , 1990
AF-T2 (CM – Pu) Chicken (21 d)	2.5 – 4.0	- BWG ↘ - RW-L,K,Pv,H ↗ (P) - RW-G ↗ - TP, albumin, uric acid ↘ - cholesterol ↘ (P) - potassium ↘ (P) - CHL ↘ (P)	- CK ↗		- hemoglobin ↘ - triglycerides ↘ - calcium ↘ - LDH, ALP ↘	- oral lesions ↗ - RW-S,Pc ↗ - RBC ↘ - magnesium ↘	- glucose ↘	- sodium →	Huff <i>et al.</i> , 1988
AF-T2 (CM – Pu) Chicken (21 d)	3.5 – 8.0	- BWG ↘ - RW-L ↗ (P) - RW-G ↗ - hemoglobin ↘ - ALP ↘	- CK ↗		- RW-Pv ↗ - RW-BF ↘ - uric acid, triglycerides, TP ↘	- RW-K,Pc ↗ - cholesterol, albumin ↘	- mortality ↗ - LDH ↘ - CHL ↗	- oral lesions ↗ - RW-H,S ↗ - GGT ↗	Kubena <i>et al.</i> , 1990
AF-T2 (CM – CM) Quail (35 d)	3.0 – 4.0	- GGT ↗ - calcium ↗			- TP, albumin, globulin ↘	- cholesterol ↘ - AST ↗ - potassium ↘	- glucose, BUN ↘ - ALT ↘	- ALP → - sodium →	Madheswaran <i>et al.</i> , 2004
AF-T2 (CM – CM) Quail (35 d)	3.0 – 4.0				- BW, feed intake ↘	- hemoglobin, RBC ↘			Madheswaran <i>et al.</i> , 2005
AF-T2 (Pu – Pu) Hamster (21 d) ¹	1.0 – 1.0	- GGT ↘	- LDH ↘ - bilirubin ↘	- ALT, AST ↘		- glucose ↘	- ALP ↗	- cholesterol ↗	Rajmon <i>et al.</i> , 2001
AF-T2 (CM – CM)	0.3 – 3.0	- RW-L,K ↗ (P) - ALT ↘		- coagulati	- BW ↘ - GGT ↗	- feed intake ↘ - mortality ↗	- RW-A ↗ - BUN ↘	- RW-G → - TP, cholesterol ↘	Raju and Devegowda,

<i>Chicken</i> (35 d)		- hemoglobin √		on time √					2000
AF-T2 (CM – CM) <i>Chicken</i> (35 d)	0.3 – 3.0	- RW-T √			- RW-BF √	- Ab IBD √	- Ab ND √		Raju and Devegowda, 2002
AF-T2 (NI – NI) <i>Rat</i> (140 d) ²	0.25 – 0.05						- RW-L,K →		Tamimi <i>et al.</i> , 1997
AF-DAS (CM – Pu) <i>Lamb</i> (34 d)	2.5 – 5.0	- TP ↗ - GGT ↗ (P)			- BWG, feed intake √ - BUN √	- CHL √		- cholesterol ↗	Harvey <i>et al.</i> , 1995a
AF-DAS (CM – Pu) <i>Pig</i> (28 d)	2.5 – 2.0				- BWG √	- ALP, GGT ↗ - AST √ - RW-S ↗	- CHL ↗ - creatinine ↗	- WBC, hemoglobin ↗ - RW-L ↗	Harvey <i>et al.</i> , 1991
AF-DAS (CM – Pu) <i>Chicken</i> (21 d)	3.5 – 5.0	- BWG √ - mortality ↗ - GGT ↗			- RW-G ↗ - LDH √	- oral lesions ↗ - RW-K,H,Pv,S,Pc ↗ - glucose, TP √	- triglycerides, albumin √ - AST √	- RW-L ↗ - cholesterol √ - calcium → - CK →	Kubena <i>et al.</i> , 1993
AF-DON (CM – NCF) <i>Pig</i> (28 d)	3.0 – 3.0	- cholesterol, glucose √ (P) - WBC ↗ (P)			- CK √ - TP √	- BWG √ - GGT, AST ↗ - BUN √ - calcium, magnesium √	- albumin √	- ALP ↗ - potassium, phosphorus √ - RBC, hemoglobin, prothrombin time ↗	Harvey <i>et al.</i> , 1989b
AF-DON (CM – NCF) <i>Chicken</i> (21 d)	2.5 – 16	- RW-Pv,G, L ↗ - ALP √			- BWG √ - hemoglobin √ - AST √	- RW-S,K ↗ - TP, albumin, uric acid, cholesterol, triglyceride √ - calcium √	- glucose √ - LDH √ - liver lipid ↗	- RBC → - phosphorus →	Huff <i>et al.</i> , 1986

1) Rajmon *et al.* (2001) : intragastrically administration, twice a week for 3 weeks 2) Tamimi *et al.* (1997) : intraperitoneally administration, twice a week for 20 weeks

- **Nature of mycotoxins** : Pu, Purified (>90%); Ex, Extracted from fungal culture material (partially purified); CM, Culture Material; NCF, Naturally Contaminated Feedstuffs; NI, No Indicated in the study

- ↗, √, → : evaluation at the end of each experiment, of the effect of the co-contaminated treatment in comparison to the control treatment.

- (P), potentiation

- unless stated, doses are expressed in mg of toxins/kg of feed. In few experiments, doses are expressed in mg of toxins/kg of body weight/day and in mg of toxins/day, and indicated as bw/d and mg/d, respectively.

- **zootechnical parameters** : BW(G), body weight (gain)

- **RW, relative weight of organs** : RW-L, liver; -K, kidney; -G, gizzard; -Pv, proventriculus; -Pc, pancreas; -H, heart; -Lg, lung; -A, adrenals; -S, spleen; -T, thymus; -BF, bursa of Fabricius.

- **biochemical and hematological parameters** : TP, total proteins; BUN, blood urea nitrogen; RBC, red blood cells; WBC, white blood cells; Sa, sphinganine; So, sphingosine; MDA, malondialdehyde; PC, protein carbonyl; CDNB, 1-chloro-2,4-dinitrobenzene; DCNB, 1,2-dichloro-4-nitrobenzene.

- **enzymatic parameters** : ALT, alanine aminotransferase; ALP, alkaline phosphatase; AST, aspartate aminotransferase; GGT, gamma-glutamyltransferase; LDH, lactate dehydrogenase; GDH, glutamate dehydrogenase; CK, creatine kinase; CHL, cholinesterase; CAT, catalase; SOD, superoxide dismutase; GSTP⁺, glutathione-S-transferase placental form positive; EROD, ethoxyresorufin *O*-dealkylase; PROD, pentoxyresorufin *O*-dephthylase
- **immune parameters** : SMC, spleen mononuclear cells; Ab, antibodies; ND, Newcastle disease; IDB, infectious bursal disease; SRBC, sheep red blood cells; DTH, delayed-type hypersensitivity.

4) Interaction between Aflatoxins (AF) and other mycotoxins

4.1) Interaction between Aflatoxins (AF) and Cyclopiazonic Acid (CPA)

The interaction between these two toxins was investigated as strains of *Aspergillus flavus* produce the toxins simultaneously. The experiments were performed on laboratory animals (rats or guinea pigs) or on chickens. The body weight gain was always decreased in animals fed the co-contaminated diet (**Table 4**). When rats were exposed for a short period to the toxins (3 days), a less than additive interaction was observed (Morrissey *et al.*, 1987). But in longer periods of exposure, the interaction between AF and CPA on the body weight of animals was qualified as synergistic or additive (Pier *et al.*, 1989; Smith *et al.*, 1992).

Biochemical and hematological alterations were also measured, and less than additive to antagonistic interaction between AF and CPA were reported (Kumar and Balachandran, 2005; Morrissey *et al.*, 1987; Smith *et al.*, 1992) (**Table 4**). Similar types of interaction were noted on immune parameters, with especially an antagonism action of CPA on the depressant effect of AF on cell-mediated immunity (Pier *et al.*, 1989) (**Table 4**). However, the author suggested that the reduced numbers of animals in the combination group, and that survivors had apparently greater resistance to the toxins may have influenced the data obtained.

Among these studies, particular attention was paid to microscopic lesions. When rats were exposed to the mycotoxins for a shorter period, less than additive or antagonistic interaction between the two toxins were described for the incidence in liver lesions (Morrissey *et al.*, 1987) (**Table 4**). Conversely, when animals were exposed for a longer period to these toxins, the interaction was suggested to be additive in liver lesions, characterized by a marked cytoplasmic vacuolation (Kumar and Balachandran, 2009; Pier *et al.*, 1989). Likewise, this greater effect was noticed in the severity and incidence of kidney lesions in broiler chickens (Kumar and Balachandran, 2009), and inconsistently in rats, depending on the ratio of mycotoxins used (Morrissey *et al.*, 1987) (**Table 4**). Of note, lesions recorded in gizzard, crop and proventriculus suggested adverse effects on the digestive tract; the interaction leading either to additive (Kumar and Balachandran, 2009) or less than additive effects (Smith *et al.*, 1992).

4.2) Interaction between Aflatoxins (AF) and - Moniliformin (MON), - Sterigmatocystin (STER), - Citrinin (CIT), - Rubratoxin (RUB)

In order to give a complete picture of the published literature, we should mention that one study investigated the combined effect of AF and moniliformin on chickens (Kubena *et al.*, 1997c), another the combined effect of AF and sterigmatocystin on guinea pigs (Richard *et al.*, 1978), and a third the combined effect of aflatoxin and citrinin on chickens (Ahamad *et al.*, 2006). The combined effect of AF and rubratoxin has also been investigated (Hayes *et al.*, 1977; Thurston *et al.*, 1989; Wyatt *et al.*, 1973). The parameters measured and the type of interaction observed between AF and MON, STER, CIT and RUB are summarized in **Table 4**.

Table 4 : Interaction between Aflatoxins (AF) and other mycotoxins

Mycotoxins (Nature) Species (exposure)	Doses	SYNERGISTIC INTERACTION			ADDITIVE INTERACTION	LESS THAN ADDITIVE INTERACTION	ANTAGONISTIC INTERACTION		REF
		Type 1	Type 2	Type 3			Type 1	Type 2	
AF-CPA (Ex – Ex) Chicken (28 d)	1.0 – 20				- RBC ↓	- albumin, glucose, hemoglobin ↓		- TP, cholesterol ↓	Kumar and Balachandra n, 2005
AF-CPA (Pu – Pu) Rat (3 d) ¹	0.1 bw/d – 0.1 bw/d					- incidence of lesions in liver ↑			Morrissey <i>et al.</i> , 1987
	2.0 bw/d – 0.1 bw/d	- mortality ↑ - incidence of lesions in kidney ↑				- BWG, feed intake ↓ - incidence of lesions in liver ↑		- glycocholic acid ↑	
	0.1 bw/d – 4.0 bw/d					- incidence of lesions in liver ↑			
	2.0 bw/d – 4.0 bw/d					- BWG, feed intake ↓ - mortality ↑ - incidence of lesions in kidney ↑ - glycocholic acid ↑	- incidence of lesions in liver ↑		
AF-CPA (Pu – Pu) Guinea pig (20 d)	0.045 – 2.2	- BWG ↓ - mortality ↑				- hemolytic complement titers ↓		- DTH reaction →	Pier <i>et al.</i> , 1989
AF-CPA (CM – Pu) Chicken (21 d)	3.5 – 50	- mortality ↑ - AST ↑ (P)			- BW ↓ - RW-L, Pc ↑	- RW-K, Pv ↑ - RW-BF ↓ - phosphorus ↓ - BUN ↑	- albumin ↓	- RW-G → - TP, cholesterol, triglycerides → - uric acid ↑ - LDH →	Smith <i>et al.</i> , 1992
AF-CIT (Ex – CM) Chicken (28 d)	0.5 – 150				- RW-K ↑	- TP ↓	- BWG, feed intake ↓ - RW-L ↑ - RBC ↓ - albumin, cholesterol ↓	- hemoglobin ↓ - uric acid ↓	Ahamad <i>et al.</i> , 2006
AF-MON (CM – CM) Chicken (21 d)	3.5 – 100	- inorganic phosphorus ↓			- BWG ↓ - RW-H ↑ - RBC ↓	- albumin ↓ - creatinine ↑ - ALT ↑	- RW-G ↑	- RW-K → - RW-BF ↓ - TP ↓ - cholesterol, calcium → - ALP ↓	Kubena <i>et al.</i> , 1997c
AF-STER	0.01 mg/d –	- albumin ↑			- BWG ↓				Richard <i>et</i>

(Ex – Pu) <i>Guinea pig</i> (14 d)	4.2 mg/d	- globulin ∇				- complement titer ∇			<i>al., 1978</i>
AF-RUB (Pu – Pu) <i>Rat</i> (30 d) ²	4.0 – 5.0	- BW ∇ (P)							<i>Hayes et al., 1977</i>
AF-RUB (Ex – Pu) <i>Guinea pig</i> (14 d) ³	0.02 bw – 8.0 bw	- complement titer, bacteriostatic activity ∇ (P) - AST ↗ (P)						- ALP ∇	<i>Thurston et al., 1989</i>
AF-RUB (CM – CM) <i>Chicken</i> (21 d)	2.5 – 500					- BW ∇ - TP ∇		- cholesterol ∇	<i>Wyatt et al., 1973</i>

1) Morrissey *et al.* (1987) : intragastrically administration 2) Hayes *et al.* (1977) : initial exposure to RUB, followed by AF on day 15 3) Thurston *et al.* (1989) : 11 administrations

- **Nature of mycotoxins** : Pu, Purified (>90%); Ex, Extracted from fungal culture material (partially purified); CM, Culture Material; NCF, Naturally Contaminated Feedstuffs; NI, No Indicated in the study

- ↗, ∇, → : evaluation at the end of each experiment, of the effect of the co-contaminated treatment in comparison to the control treatment.

- (P), potentiation

- unless stated, doses are expressed in mg of toxins/kg of feed. In few experiments, doses are expressed in mg of toxins/kg of body weight/day and in mg of toxins/day, and indicated as bw/d and mg/d, respectively.

- **zootechnical parameters** : BW(G), body weight (gain)

- **RW, relative weight of organs** : RW-L, liver; -K, kidney; -G, gizzard; -Pv, proventriculus; -Pc, pancreas; -H, heart; -Lg, lung; -A, adrenals; -S, spleen; -T, thymus; -BF, bursa of Fabricius.

- **biochemical and hematological parameters** : TP, total proteins; BUN, blood urea nitrogen; RBC, red blood cells; WBC, white blood cells; Sa, sphinganine; So, sphingosine; MDA, malondialdehyde; PC, protein carbonyl; CDNB, 1-chloro-2,4-dinitrobenzene; DCNB, 1,2-dichloro-4-nitrobenzene.

- **enzymatic parameters** : ALT, alanine aminotransferase; ALP, alkaline phosphatase; AST, aspartate aminotransferase; GGT, gamma-glutamyltransferase; LDH, lactate dehydrogenase; GDH, glutamate dehydrogenase; CK, creatine kinase; CHL, cholinesterase; CAT, catalase; SOD, superoxide dismutase; GSTP⁺, glutathione-S-transferase placental form positive; EROD, ethoxyresorufin *O*-dealkylase; PROD, pentoxyresorufin *O*-dephthylase

- **immune parameters** : SMC, spleen mononuclear cells; Ab, antibodies; ND, Newcastle disease; IDB, infectious bursal disease; SRBC, sheep red blood cells; DTH, delayed-type hypersensitivity.

II. INTERACTIONS BETWEEN FUSARIOTOXINS

1) Interaction between Fumonisin (FB) and other Fusariotoxins

1.1) Interaction between FB and MON

Several studies reported the effects of interaction between FB and MON, and mainly with highly contaminated feeds and in poultry (**Table 5**).

A less than additive and an antagonistic interactions in turkeys (Bermudez *et al.*, 1997; Li *et al.*, 2000) and in broiler chicks (Ledoux *et al.*, 2003), respectively, were observed on the growth animals depression. In pigs, quails and catfish, the effect of the multi-contaminated diet on BWG ranged from less than additive to additive and to synergistic (Harvey *et al.*, 2002; Sharma *et al.*, 2008; Yildirim *et al.*, 2000) (**Table 5**). However, the interaction on feed consumption was not consistently associated with the interaction on BWG.

Some authors reported animal mortality, especially due to MON presence in chickens (Ledoux *et al.*, 2003). Surprisingly, no mortality in laying hens fed combination treatment has been observed compared to individual treatments (Kubena *et al.*, 1999). But as suggested by the authors, the higher mortality in FB group alone may be attributed to the increased egg weights because 4 of the deaths were attributed to uterine prolapses in the later stages of production. In the same study, antagonistic interaction between MON and FB was reported, with a sparing action of MON on the FB effects on eggs performance (**Table 5**).

Interestingly, in comparison to other mycotoxin interactions both FB and MON induced an increase of serum TP and albumin concentrations. Other mycotoxins mainly reported a decrease of these biochemical compounds, likely due to their property to inhibit the synthesis of proteins.

Serum AST concentrations increased following exposure to the combined treatment, but different types of interaction, from synergism type 2 to antagonism were recorded (Bermudez *et al.*, 1997; Harvey *et al.*, 2002; Kubena *et al.*, 1999; Ledoux *et al.*, 2003; Sharma *et al.*, 2008) (**Table 5**). Of note, Yildirim *et al.* (2000) analyzed serum pyruvate concentrations in animals exposed to contaminated diets, as MON is known to inhibit pyruvate dehydrogenase. At the higher inclusion of FB in the co-contaminated diet, he reported a synergistic interaction on the level of serum pyruvate (**Table 5**), suggesting a stronger inhibition of this enzyme.

This enzyme inhibition could be related in the increase of relative weight of hearts in animals exposed to MON, as this enzyme is involved in the production of ATP and that the heart may have to increase the blood flow to supply more oxygen to the body in order to increase ATP production (Ledoux *et al.*, 2003). Cardiomegaly was thus observed, and MON combined with FB led either to synergistic and additive effects (Kubena *et al.*, 1999; Ledoux *et al.*, 2003) or less than additive effects (Bermudez *et al.*, 1997; Ledoux *et al.*, 2003) (**Table 5**). A lack of cardiomegaly was indicated by Harvey *et al.* (2002), showing that swine are less sensitive to MON than poultry.

Cardiac lesions, mainly characterized by a diffuse loss of cardiomyocyte cross striations were recorded in the MON and MON+FB groups, with the same degree of severity between these groups (Bermudez *et al.*, 1997; Harvey *et al.*, 2002; Ledoux *et al.*, 2003). Similarly, liver lesions described by these authors were attributed to FB and were not more severe in the FB+MON diet. On the other hand, combined mycotoxins revealed smaller hepatocellular nuclei in fish, indicating an additive or synergistic interaction according to the concentration of FB in the combination (Yildirim *et al.*, 2000).

Two studies focused on the interaction effect on the immune system (Li *et al.*, 2000; Sharma *et al.*, 2008) (**Table 5**). An additive effect was observed in the reduced antibodies against Newcastle Disease. Li *et al.* (2000) indicated that after an *E. coli* challenge, the ability of animals to eliminate bacteria from the blood system was diminished in animals exposed to

mycotoxins. However, a less than additive interaction was reported in the multi-contaminated diet, as shown by the increased numbers of bacterial colonies in blood and tissues. Likewise in this study, a less than additive effect was reported in the reduced proliferation of lymphocytes upon mitogenic activation. By contrast, Sharma *et al.* (2008) observed that MON reversed the immunosuppressive effects of FB, in quails undergoing skin hypersensitivity test, suggesting an antagonistic interaction on the cellular immune response.

1.2) Interaction between FB and TCT

1.2.1) with TCT type A

Interaction between FB and T-2 toxin was reported in turkeys and in chickens (Kubena *et al.*, 1997a; Kubena *et al.*, 1995a) (**Table 5**).

Both experiments reported an additive effect on the BWG depression. Oral lesions, caused by T-2 toxin, were also recorded, but effect on scores differed between studies. The antagonist effect was likely due to the greater reduction in feed consumption, thereby diminishing the T-2 toxin ingestion in the combination group (Kubena *et al.*, 1997a). In both studies, the increased relative weight of gizzard (RW-G) was related to the irritant property of T-2 toxin, typically described as focally reddened mucosa (Kubena *et al.*, 1995a).

The decreased or increased effect on cholesterol concentration reflects the individual effect of FB in each study. An important difference between these two studies is the activity of hepatic enzymes. On one hand, T-2 toxin seems to potentiate the FB effect (Kubena *et al.*, 1995a) on AST and LDH, whereas in the experiment of Kubena *et al.* (1997a), the effect on these enzymes was lesser than FB alone. No histological analysis on liver was done to find out a possible link, but a partial explanation could also be due to the greater reduction in feed intake in the combination group (Kubena *et al.*, 1997a). Another reason may be the type of birds used and the sex, sensitivity being different between male and female.

However, the likelihood of encountering concentrations of 300 mg FB/kg in finished feed is very small.

One study investigated the interaction of DAS with a very high content of FB in diets (Kubena *et al.*, 1997b), and reported additive effect on performance and antagonistic effect for oral lesions (**Table 5**). Hematological and biochemical values showed different types of interaction, from synergism to antagonism.

1.2.2) with TCT type B

Three experiments, performed in pigs and in chickens, investigated the combined effect of DON and FB, the most frequently detected fusariotoxins (Grenier *et al.*, 2011; Harvey *et al.*, 1996; Kubena *et al.*, 1997a) (**Table 5**).

The type of interaction on body weight gain differed depending on the experiment. A synergistic interaction was reported by Harvey, no change in this parameter whatever the toxins included in the diet was observed by Grenier. In chickens, Kubena *et al.* (1997a) observed a less than additive effect, mainly due to FB in the combination group. Interestingly, in this latter study, feed intake was slightly increased in comparison to the other treatments (**Table 5**).

An antagonistic interaction has been observed for albumin. DON in the multi-contaminated diet seems to potentiate the FB effects, as reflected by the hepatic enzymes concentrations (Harvey *et al.*, 1996; Kubena *et al.*, 1997a). It could be linked to the unexpected effect on the RW-L in the experiment conducted by Harvey *et al.* (1996), where the combination led to an

opposite effect compared to individual effects (**Table 5**). The large and synergistic decrease in the RW-L may be due to an important hepatic damage, as already mentioned in this review for AF-FB interaction. However, histologically in this experiment, hepatic lesions observed (necrosis) in animals fed the combined mycotoxins, were not more severe than those observed in animals fed the FB diet. By contrast, Grenier *et al.* (2011) showed a greater effect in the severity and extent of lesions (mainly hepatocytes vacuolization and megalocytosis) (**Table 5**), suggesting that DON, known to alter the intestinal permeability, had enhanced the intestinal absorption of FB, well known to be poorly absorbed. The differences in these two studies are most likely due to the different concentrations of FB used. These two studies are in agreement regarding the kidney lesions, where pigs exposed to the DON+FB diet did not have an increased frequency of lesions compared to individual diets (**Table 5**).

The immune system was also evaluated following the exposure to both DON and FB (**Table 5**). Harvey *et al.* (1996) recorded a potentiation of the effect of FB by DON in the reduced lymphocytes proliferation upon mitogenic stimulation. Grenier *et al.* (2011) reported a less than additive effect on the decreased index when lymphocytes were stimulated by ovalbumin. In their experiment, pigs were immunized with ovalbumin and the specific immune response was thus impaired, as also displayed by the additive effect on the content of specific IgG. In contrast, an antagonistic interaction was recorded in the levels of specific IgA, showing a sparing effect of FB on the DON-induced IgA elevation. Also in this study, an additive effect on the decreased expression of cytokines in spleen was noted.

1.3) Interaction between FB and Fusaric Acid (FA)

A synergistic interaction was reported when FB and Fusaric Acid (FA) were combined in equal concentrations and injected into chicken eggs (**Table 5**), resulting in increased toxicity, as shown by the percentage of dead embryos (Bacon *et al.*, 1995). In the same study, when a relatively non-toxic concentration of FA was combined with graded doses of FB, a synergistic response was also obtained.

2) Interaction between Trichothecenes (TCT)

2.1) TCT type A

One study investigated the interaction between the two type A trichothecenes, T-2 toxin and DAS. Both toxins inhibit protein synthesis in eukaryotic cells and are highly toxic to poultry (Diaz *et al.*, 1994). In laying hens, Diaz *et al.* (1994) observed an additive effect of T-2 toxin and DAS on feed intake and oral lesions (**Table 5**). In the experiment, the authors recorded egg production throughout the trial. The lower egg production observed in hens receiving either T-2 toxin or DAS recovered gradually during the experiment. Interestingly, the decrease in egg production of hens fed the co-contaminated diet became progressively worse (**Table 5**). The authors suggested that whereas hens exposed to a single mycotoxin at low concentrations may be able to sustain a satisfactory rate of egg production, they may not be able to tolerate a combination of mycotoxins at similarly low concentrations.

2.2) TCT type A and B

Two reports focused on the interaction between type B and type A trichothecenes, DON and T-2 toxin (Friend *et al.*, 1992; Kubena *et al.*, 1989b). In swine, except for the highest dose of T-2 toxin that interact synergistically with DON in the decreased BWG and feed intake, a less than additive effect was observed on these zootechnic parameters following the combination exposure, reflecting predominantly the DON effect (Friend *et al.*, 1992) (**Table 5**). By contrast, in broiler chicks, most of the observed effects (oral lesions, total protein,

albumin and LDH) were due to T-2 toxin in the combined treatment (Kubena *et al.*, 1989b). However, an additive effect was obtained on the body weight gain and on the serum cholesterol concentration (**Table 5**). Decreased cholesterol levels could suggest inhibition of biosynthesis, with liver involvement and perhaps a shift of concentration from blood to the liver.

2.3) TCT type B

One study investigated the interaction between the two type B trichothecenes, deoxynivalenol and nivalenol (NIV), using two different low doses of both toxins (Gouze *et al.*, 2005) (**Table 5**). Although the lowest dose of NIV and the two doses of DON used did not affect the plasma levels of uric acid, when used in combination the toxins act in a synergistic manner. Whatever the dose of NIV and DON used, when the toxins were used in combination an additive effect was observed for the total protein level.

Ingestion of DON or NIV induces IgA nephropathy in mice. Depending on the doses of two toxins, when used in combination the interaction range from synergistic to additive or less than additive on IgA synthesis. The hepatic drug metabolism activity was also assessed. When mice were exposed to DON and NIV, the toxins showed an antagonistic interaction on the activities of two monooxygenase (EROD and PROD). By contrast, for the highest doses of DON and NIV, a synergistic effect was observed on the elevated activity of glutathione S-transferase (DCNB as substrates).

3) Interaction between Deoxynivalenol (DON) and other Fusariotoxins

3.1) Interaction between DON and Zearalenone (ZEA)

Three publications investigated the interaction between DON and zearalenone (ZEA) (Boeira *et al.*, 2000; Forsell *et al.*, 1986; Pestka *et al.*, 1987). Forsell *et al.* (1986) showed in mice that exposure to the combined treatment resulted mainly in an antagonistic interaction (**Table 5**). The author observed an antagonistic effect of ZEA on the DON-induced elevation of serum IgA. Similarly, antagonism was noted in the ability of DON to inhibit the delayed hypersensitivity response in presence of ZEA (Pestka *et al.*, 1987). However, the resistance to *Listeria monocytogenes* was reduced in an additive manner by co-administration of DON and ZEA (Pestka *et al.*, 1987) (**Table 5**).

Interestingly, DON+ZEA combination was assessed on growth of brewing yeast, considering that grains contaminated with mycotoxins are used for beer production, and may be introduced at different steps of the brewing process (Boeira *et al.*, 2000). Despite the effect caused by combination of DON and ZEA at high concentrations, shown to pass from antagonism to synergism depending on the ratio of toxins in the mixture, when low concentrations were used, inhibition of yeast growth was not observed.

3.2) Interaction between DON and MON

Due to the relatively tolerance of DON in poultry, its interaction with MON reflected mostly the MON effects (Harvey *et al.*, 1997; Morris *et al.*, 1999). Poults fed diets containing MON alone and the DON-MON combination exhibited an increased incidence of variable sized cardiomyocyte nuclei, with numerous large, giant nuclei and a generalized loss of cardiomyocyte cross striations (Morris *et al.*, 1999). Isolated renal tubules in sections of kidney were noted to have diffuse mineralization in animals fed MON and DON-MON (Harvey *et al.*, 1997; Morris *et al.*, 1999), and also extensive tubular epithelial degeneration (Harvey *et al.*, 1997). But Harvey *et al.* (1997) indicated a moderation of the severity of lesions in the tissues of chicks fed DON-MON, suggesting an antagonistic effect.

Table 5 : Interaction between Fusariotoxins

Mycotoxins (Nature) Species (exposure)	Doses	SYNERGISTIC INTERACTION			ADDITIVE INTERACTION	LESS THAN ADDITIVE INTERACTION	ANTAGONISTIC INTERACTION		REF
		Type 1	Type 2	Type 3			Type 1	Type 2	
FB-MON (CM – CM) Turkey (21 d)	200 – 100	- feed intake ↓ (P)			- RW-L ↑ - AST ↑	- BWG ↓ - RW-H ↑ - RW-BF ↓	- LDH ↑		Bermudez <i>et al.</i> , 1997
FB-MON (CM – CM) Pig (28 d)	100 – 100	- feed intake ↓ - creatinine ↑ (P)			- RBC ↓	- BWG ↓ - mortality ↑ - glucose ↓	- inorganic phosphorus ↓	- AST, ALP, LDH, GGT ↑ - total iron →	Harvey <i>et al.</i> , 2002
FB-MON (CM – CM) Laying hen (420 d)	100 – 50	- RW-H ↑ (P) - albumin ↓	- AST ↑	- mortality ↓		- CK ↓	- RW-L,K ↓	- uric acid → - egg production ↓ - egg weight →	Kubena <i>et al.</i> , 1999
FB-MON (CM – CM) Chicken (21 d) ¹	100 – 100				- RW-H ↑		- feed intake ↓ - mortality ↑ - AST ↑	- BWG ↓	Ledoux <i>et al.</i> , 2003
	100 – 200					- RW-H,K,L ↑ - albumin, TP ↑	- mortality ↑ - AST ↑		
	200 – 100	- RW-L ↑ - albumin, TP ↑			- RW-K ↑	- RW-H ↑	- feed intake, BWG ↓ - mortality ↑ - AST ↑		
	200 – 200				- RW-K,L ↑	- RW-H ↑ - albumin ↑	- mortality ↑ - AST ↑	- TP ↑	
FB-MON (CM – CM) Turkey (21-28 d)	200 – 100				- Ab ND ↓	- feed intake, BWG ↓ - RW-T,BF,S ↓ - lymphocytes stimulation ↓ - bacteria in tissue & blood, mortality p.i ↑			Li <i>et al.</i> , 2000
FB-MON (CM – CM) Quail (35 d)	200 – 100				- BW ↓ - mortality ↑	- TP, cholesterol ↑ - ALT, AST ↑	- albumin ↑ - LDH, CK ↑	- creatinine → - DTH reaction →	Sharma <i>et al.</i> , 2008
FB-MON	20 – 40				- size of hepatocyte nuclei ↓	- BWG, feed intake ↓ - Sa/So liver ↑			Yildirim <i>et</i>

(CM – CM) <i>Fish</i> (70 d)	40 – 40	- BWG ↓ - serum pyruvate ↑ - size of hepatocyte nuclei ↓			- feed intake ↓	- Sa/So liver ↑			<i>al., 2000</i>
FB-T2 (CM – Pu) <i>Turkey</i> (21 day)	300 – 5.0	- RW-L ↑ (P) - RW-G ↑ - RBC, hemoglobin ↑ - AST ↑ - LDH ↑ (P)			- BWG ↓ - inorganic phosphorus ↓	- oral lesions ↑ - RW-Pc ↑ - cholesterol ↓	- uric acid ↑		<i>Kubena et al., 1995a</i>
FB-T2 (CM – Pu) <i>Chicken</i> (19 d)	300 – 5.0	- RW-Pc ↑ (P)			- BWG, feed intake ↓ - mortality ↑ - RW-G ↑	- RW-L,K ↑ - cholesterol ↑ - calcium ↑		- oral lesions ↑ - RW-S → - TP, albumin ↑ - AST, LDH, GGT ↑	<i>Kubena et al., 1997a</i>
FB-DAS (CM – Pu) <i>Turkey</i> (21 d)	300 – 4.0	- inorganic phosphorus ↓ - RBC ↑ (P)			- BWG, feed intake ↓	- RW-L,G ↑ - RW-S,H ↓ - AST, LDH ↑	- triglycerides ↓ - uric acid ↑	- oral lesions ↑ - RW-Pc → - cholesterol ↓	<i>Kubena et al., 1997b</i>
FB-DON (CM – NCF) <i>Pig</i> (28 d)	50 – 4.0	- BWG ↓ - AST, CHL, ALP ↑ (P) - lymphocytes stimulation ↓ (P)		- RW-L ↓	- feed intake ↓ - RW-Lg ↑	- creatinine ↑	- albumin ↓	- cholesterol ↑ - GGT ↑	<i>Harvey et al., 1996</i>
FB-DON (CM – NCF) <i>Chicken</i> (21 d)	300 – 15	- mortality ↑ - RW-Pv ↑ (P) - cholesterol ↑ (P) - AST ↑ (P) - LDH ↑	- feed intake ↑		- RW-G ↑ - BUN ↑ - GGT ↑	- BWG ↓ - RW-L,K ↑ - TP ↑	- RW-H →	- RW-BF →	<i>Kubena et al., 1997a</i>
FB-DON (CM – Ex) <i>Pig</i> (35 d)	6.0 – 3.0				- severity, extent of liver & lung lesions ↑ - specific Ab IgG ↓ - cytokines expression ↓	- neutrophils ↓ - severity, extent of kidney lesions ↑ - specific lymphocytes stimulation ↓		- creatinine, albumin → - specific Ab IgA →	<i>Grenier et al. (2011)</i>
FB-FA (Pu – Pu) <i>Chicken egg</i> (21 d)	1.0 – 1.0 (μg/egg)	- embryos mortality ↑							<i>Bacon et al., 1995</i>
	5.0 – 5.0 (μg/egg)	- embryos mortality ↑							
	25 – 25 (μg/egg)	- embryos mortality ↑							
	50 – 50 (μg/egg)	- embryos mortality ↑							
T2-DAS (Pu – Pu) <i>Laying hen</i> (24 d)	2.0 – 2.0	- egg production (at end of the trial) ↓			- oral lesions ↑ - feed intake ↑	- LDH ↑	- egg production (at intermediate time) ↓ - GDH ↓		<i>Diaz et al., 1994</i>

DON-T2 (CM – Pu) <i>Pig</i> (35 d)	2.5 – 0.4					- BWG, feed intake ↓			Friend <i>et al.</i> , 1992
	2.5 – 0.8					- BWG, feed intake ↓			
	2.5 – 1.6					- BWG, feed intake ↓			
	2.5 – 3.2	- BWG, feed intake ↓							
DON-T2 (NCF – Pu) <i>Chicken</i> (21 d)	16 – 4.0				- BW ↓ - cholesterol ↓	- RW-G,BF ↑ - TP, albumin ↓ - LDH ↓ - oral lesions ↑			Kubena <i>et al.</i> , 1989b
DON-NIV (Pu – Pu) <i>Mouse</i> (28 d) ²	0.071 bw – 0.071 bw	- uric acid ↑			- TP ↑ - PROD ↑	- IgA ↑ - CDNB ↑	- total CO2 ↓ - EROD ↑		Gouze <i>et al.</i> , 2005
	0.071 bw – 0.355 bw	- uric acid ↑ (P)			- TP ↑ - IgA ↑ - CDNB ↑	- total CO2 ↓ - PROD ↑ - DCNB ↑	- EROD ↑	- feed intake ↓	
	0.355 bw – 0.071 bw	- phosphorus ↑ - uric acid ↑			- total CO2 ↓ - TP ↑	- IgA ↑ - CDNB ↑	- EROD, PROD ↑		
	0.355 bw – 0.355 bw	- IgA ↑ - DCNB ↑			- TP ↑ - phosphorus ↑	- total CO2 ↓ - uric acid ↑ - CDNB ↑	- EROD, PROD ↑	- feed intake ↓	
DON-ZEA (Pu – Pu) <i>Mouse</i> (56 d)	5.0 – 10			- RBC ↓			- BW ↓ - RW-L → - IgA ↑		Forsell <i>et al.</i> , 1986
DON-ZEA (Pu – Pu) <i>Mouse</i> (14-21 d)	25 – 10				- bacteria in spleen ↑			- DTH reaction ↓	Pestka <i>et al.</i> , 1987
DON-MON (Ex – CM) <i>Turkey</i> (21 d)	20 – 100		- feed intake ↓ - globulin ↓		- RW-K ↑	- BWG ↓		- RW-H ↑ - calcium →	Morris <i>et al.</i> , 1999

1) Ledoux *et al.* (2003) : for the highest dose of MON in the combination, feed intake and BWG not took into account due to high mortality 2) Gouze *et al.* (2005) : administration thice a week for 4 weeks

- **Nature of mycotoxins** : Pu, Purified (>90%); Ex, Extracted from fungal culture material (partially purified); CM, Culture Material; NCF, Naturally Contaminated Feedstuffs; NI, No Indicated in the study

- ↑, ↓, → : evaluation at the end of each experiment, of the effect of the co-contaminated treatment in comparison to the control treatment.

- (P), potentiation

- unless stated, doses are expressed in mg of toxins/kg of feed. In few experiments, doses are expressed in mg of toxins/kg of body weight/day and in mg of toxins/day, and indicated as bw/d and mg/d, respectively.

- **zootechnical parameters** : BW(G), body weight (gain)

- **RW, relative weight of organs** : RW-L, liver; -K, kidney; -G, gizzard; -Pv, proventriculus; -Pc, pancreas; -H, heart; -Lg, lung; -A, adrenals; -S, spleen; -T, thymus; -BF, bursa of Fabricius.

- **biochemical and hematological parameters** : TP, total proteins; BUN, blood urea nitrogen; RBC, red blood cells; WBC, white blood cells; Sa, sphinganine; So, sphingosine; MDA, malondialdehyde; PC, protein carbonyl; CDNB, 1-chloro-2,4-dinitrobenzene; DCNB, 1,2-dichloro-4-nitrobenzene.

- **enzymatic parameters** : ALT, alanine aminotransferase; ALP, alkaline phosphatase; AST, aspartate aminotransferase; GGT, gamma-glutamyltransferase; LDH, lactate dehydrogenase; GDH, glutamate dehydrogenase; CK, creatine kinase; CHL, cholinesterase; CAT, catalase; SOD, superoxide dismutase; GSTP⁺, glutathione-S-transferase placental form positive; EROD, ethoxyresorufin *O*-dealkylase; PROD, pentoxyresorufin *O*-dephthylase

- **immune parameters** : SMC, spleen mononuclear cells; Ab, antibodies; ND, Newcastle disease; IDB, infectious bursal disease; SRBC, sheep red blood cells; DTH, delayed-type hypersensitivity.

III. INTERACTIONS BETWEEN OCHRATOXIN A AND OTHER MYCOTOXINS

1) Interaction between OTA and Citrinin (CIT)

Regarding zootechnical parameters, Manning *et al.* (1985) observed an antagonistic interaction for body weight gain when chickens were fed with OTA and CIT (**Table 6**). This response was most likely the result of the improved feed intake in the OTA+CIT group. Birds receiving the co-contaminated feed also showed an antagonistic effect in the increase of water consumption that is normally associated with CIT toxicosis.

Changes in serum total protein, cholesterol, triglycerides, and uric acid concentration also demonstrated an antagonistic effect between the two toxins (**Table 6**), and suggested that the combination of mycotoxins gave some advantage to the birds and allowed them to maintain biochemical parameters close to the to normal ranges. A proposed mechanism would be that the cytoplasmic accumulation of smooth endoplasmic reticulum may be indicative of enzyme induced by the exposure to CIT and would protect against the toxicity of OTA (Manning *et al.* 1985; Brown *et al.* 1986).

Less than additive and antagonistic interactions between the two toxins were also observed in rabbits for lymphocytes proliferation and specific antibody response (Kumar *et al.*, 2008) (**Table 6**).

Because both OTA and CIT target the kidney and have been implicated as causal agents for the Balkan endemic nephropathy, most of the experiments conducted with both OTA and CIT investigated their effects on renal function and structure (Brown *et al.*, 1986; Glahn *et al.*, 1988; Kitchen *et al.*, 1977a; b; Kumar *et al.*, 2007; Manning *et al.*, 1985; Siraj *et al.*, 1981). A synergistic interaction between the two toxins was observed in dogs when looking at the severity of clinical signs and mortality (Kitchen *et al.*, 1977a). Concentrations of urinary GOT and LDH were significantly increased in animals exposed to both toxins, signaling renal damage. A synergistic interaction was also observed for the inhibition of the renal Na⁺-K⁺-ATPase activity in neonatal rats (Siraj *et al.*, 1981) (**Table 6**). An experiment also reported that the OTA pretreatment blunted the diuretic effect of CIT on kidney of pullets (Glahn *et al.*, 1988). But as indicated by the author, the sequential use of toxins and mode of administration may create conditions that differ significantly from situations in which OTA and CIT are present simultaneously.

Ultrastructural assessment of kidney showed mainly degenerative and necrotic changes in the proximal and distal tubules. The nephrotoxicity was related with mitochondria damage (Kumar *et al.* 2007; Brown *et al.* 1986). In the animal group receiving both toxins, these lesions were more severe and intense (Kitchen *et al.*, 1977b; Kumar *et al.*, 2007), similar to the ones observed in the OTA-exposed group (Brown *et al.*, 1986; Manning *et al.*, 1985) or less severe than the ones observed in the animal group receiving the highest dose of CIT alone (Kitchen *et al.*, 1977b).

An increased genotoxicity of OTA was also observed in the presence of CIT, with increased DNA-adducts in kidney, suggesting synergism (Pfohl-Leszkowicz *et al.*, 2008) (**Table 6**).

Two studies investigated the interaction between OTA and CIT on teratogenesis led to different results. Mayura *et al.* (1984) observed synergism between the toxins when looking at fetal malformations in rats (**Table 6**). By contrast, Vesela *et al.* (1983) observed the same teratogenic effects in chicken embryos exposed to either OTA alone or OTA+CIT.

2) Interaction between OTA and Fusariotoxins

2.1) Interaction between OTA and TCT

2.1.1) with TCT type A

Five studies focused on the interaction between OTA and T-2 toxin (Garcia *et al.*, 2003; Harvey *et al.*, 1994; Kubena *et al.*, 1989a; Raju and Devegowda, 2000; 2002) (**Table 6**).

Except for the study of Raju and Devegowda (2000), the experiments showed that the association of OTA and T-2 toxin act in an additive manner to depress the body weight gain (**Table 6**). The serum concentrations of albumin, creatinine, cholesterol, uric acid and phosphorus were affected in these studies, representing mostly the effect of OTA alone, but less than additive or antagonistic interactions were reported. These studies noted an additive interaction on the decreased hemoglobin concentration. Effect of both OTA and T-2 toxin ingestion on enzyme concentrations induced different type of interactions between these studies, but in most of the case a significant decrease was reported (**Table 6**). It was suggested that the property to inhibit the protein synthesis by these mycotoxins was involved.

It seems from these studies that T-2 toxin may interfere in the OTA-induced renal impairment, as shown by the antagonistic interaction on the RW-K, uric acid, phosphorus or also the percentage of necrotic tubular cells (**Table 6**). Nonetheless, microscopic lesions showing degenerative changes in renal tubular epithelium and proximal convoluted tubules were similarly reported for OTA and OTA+T2 groups (Harvey *et al.*, 1994; Kubena *et al.*, 1989a). In liver, T-2 toxin appeared to slightly potentiate the hepatotoxicity of OTA, visible on the percentage of repairing tissue (Garcia *et al.*, 2003) (**Table 6**).

Finally, their interaction on immune system was reported by Harvey *et al.* (1994) and Raju and Devegowda (2002). Although an additive effect was observed on the lymphocytes proliferation upon mitogenic stimulation, the reduced activity of phagocytosis in the combined treatment was similar to the individual OTA treatment (Harvey *et al.*, 1994), and an antagonistic effect was reported for the level of specific antibodies (Raju and Devegowda, 2002) (**Table 6**).

One experiment investigated the interaction between OTA and DAS in chicken (**Table 6**), and observed in general less than additive or antagonistic effect (Kubena *et al.*, 1994a). Surprisingly, no mortality was recorded in comparison to the individual treatments. A less than additive effect was observed for the oral lesions, induced by DAS. Like T-2 toxin, in association with OTA, DAS seems to spare the OTA-induced renal dysfunction, as shown with the antagonistic effect on uric acid concentration.

2.1.2) with TCT type B

One study focused on the interaction between OTA and DON in chicken (Kubena *et al.*, 1988). For many parameters, such as BWG, relative weight of organs and biochemistry, the interaction was less than additive or antagonistic in nature (**Table 6**). Similarly, lesions in liver and kidney were only due to the OTA presence in the contaminated diet.

2.2) Interaction between OTA and FB

Three experiments were conducted in rats, turkey poults and rabbits to analyze the interaction between these two toxins (**Table 6**); however the authors used very different doses of mycotoxins (Domijan *et al.*, 2007; Kubena *et al.*, 1997b; Sivakumar *et al.*, 2009). Using very high concentrations of FB, Kubena *et al.* (1997b) showed synergistic effects in association with OTA compared to mycotoxins alone (performance, some biochemical parameters and enzyme levels). Using lower doses of toxins, Sivakumar *et al.* (2009) observed less than additive or additive effects on biochemistry and enzyme levels. Interestingly, in both studies, the concentrations of serum enzymes were increased compared to the interaction between OTA and T-2 toxin (**Table 6**).

Using doses of toxins reflecting the European-type diet, Domijan *et al.* (2007) analyzed the interaction between these two toxins on the oxidative stress, in liver and kidneys of rats (**Table 6**). They observed that although the lowest doses of OTA and FB given separately did not increase the concentration of malondialdehyde (MDA) and protein carbonyls (PCs) in the liver, their combination produced a synergistic effect. Similarly, in the kidney, the combination further increased the PCs concentration, ranging from synergistic to additive or to less than additive effect, depending on the doses used. The catalase activity in rat kidney decreased in a synergistic manner after exposure to both OTA and FB, while this parameter was not affected by separate OTA or FB treatments.

2.3) Interaction between OTA and ZEA

Halabi *et al.* (1998) studied the histopathological effects of OTA and ZEA, in liver and kidneys of rats. Small amounts of mycotoxins, over a long period were given intraperitoneally to rats. The authors indicated that ZEA antagonizes the toxic effects of OTA for body weight gain and relative weight of kidney, leading to no changes in the combination group. Likewise, less severe lesions in kidneys were observed for ZEA and ZEA+OTA in comparison to the severity induced by OTA.

3) Interaction between OTA and *Aspergillus/Penicillium* toxins

3.1) Interaction between OTA and Penicillic Acid (PA)

Combination of OTA and PA showed less than additive effects on body weight in chickens (Kubena *et al.*, 1984) and in mice (Shepherd *et al.*, 1981), but act in a synergistic manner in the increase of the mortality in both species (**Table 6**). Shepherd *et al.* (1981) also indicated that the combination produced more extensive lesions in kidney within the proximal convoluted tubules, but with less renal damage at day 21 than at day 10, showing a recovery from the initial shock.

3.2) Interaction between OTA and CPA

In chickens, synergistic interactions were often recorded after combination of both mycotoxins (**Table 6**), and some of the deleterious effects induced by OTA were exacerbated by the presence of CPA (Gentles *et al.*, 1999).

Another interaction, that cannot be classified in these different sections, concern the association between T-2 toxin and CPA. This interaction was investigated in chickens either on general parameters (Kubena *et al.*, 1994b) or on immune parameters (Kamalavenkatesh *et al.*, 2005) (**Table 6**). Kubena *et al.* (1994b) showed less than additive effects on animal performances and synergistic effects on relative weight of liver and kidney, as well as lipid compounds. Kamalavenkatesh *et al.* (2005) reported some additive interactions in the reduced subpopulations of lymphocytes in lymphoid organs, and in the lymphocytes stimulation. By contrast, he observed an antagonistic interaction in the specific antibodies content.

Table 6 : Interaction between Ochratoxin A (OTA) and other mycotoxins; interaction between T-2 toxin (T2) and cyclopiazonic acid (CPA) is also included

Mycotoxins (Nature) Species (exposure)	Doses	SYNERGISTIC INTERACTION			ADDITIVE INTERACTION	LESS THAN ADDITIVE INTERACTION	ANTAGONISTIC INTERACTION		REF
		Type 1	Type 2	Type 3			Type 1	Type 2	
OTA-CIT (Pu – Pu) Rabbit (60 d)	0.75 – 15					- lymphocytes stimulation √	- Ab SRBC √		Kumar <i>et al.</i> , 2008
OTA-CIT (Pu – Pu) Chicken (21 d)	3.0 – 300				- RW-L √ - potassium √	- RW-K √ - glucose √ - calcium √ - lesion score in kidney √	- feed intake, BW √ - water consumption √ - triglycerides √ - phosphorus √	- TP, albumin, globulin √ - cholesterol → - uric acid √	Manning <i>et al.</i> , 1985
OTA-CIT (Pu – Pu) Rat (20 d) ¹	1.0 – 30	- maternal mortality √ - resorptions √ - live fetuses √ - % malformed √							Mayura <i>et al.</i> , 1984
OTA-CIT (Pu – Pu) Rat (21 d)	0.026 – 0.1	- DNA adducts in kidney √ (P)						- BWG →	Pfohl-Leszkowicz <i>et al.</i> , 2008
OTA-CIT (Pu – Pu) Rat (1 d) ²	1.0 – 25	- renal Na ⁺ -K ⁺ -ATPase activity √	- NADPH dehydrogenase, NADPH cytochrome c reductase √	- cytochrome P-450 content √		- renal Mg ²⁺ -ATPase activity √	- aniline oxidation √		Siraj <i>et al.</i> , 1981
OTA-T2 (CM – CM) Chicken (21 d)	0.567 – 0.927	- % repairing tissue in liver √			- BWG, feed intake √	- TP, albumin, globulin √ - % tumid, necrotic cells in liver √	- RW-L √	- uric acid → - RW-K √ - % necrotic tubular cells in kidney √	Garcia <i>et al.</i> , 2003
OTA-T2 (Pu – Pu) Pig (30 d)	2.5 – 8.0	- GGT √			- BWG, feed intake √ - hemoglobin √ - lymphocytes stimulation √	- cholesterol √ - creatinine √ - ALP √ - phagocytosis activity √	- inorganic phosphorus √	- TP →	Harvey <i>et al.</i> , 1994
OTA-T2 (Pu – Pu) Chicken	2.0 – 4.0	- RW-G √ - BUN √ - triglycerides √		- calcium →	- BWG √ - hemoglobin √ - TP √	- oral lesions √ - RW-L,K,Pv √ - creatinine √	- phosphorus √	- RW-Pc √ - magnesium √ - uric acid √	Kubena <i>et al.</i> , 1989a

(21 d)		- AST ↓ (P)			- CK ↓ - LDH ↓	- cholesterol, albumin ↓		- GGT → - ALP ↓	
OTA-T2 (CM – CM) <i>Chicken</i> (35 d)	2.0 – 3.0				- RW-G ↑ - hemoglobin ↓	- BW, feed intake ↓ - mortality ↑ - RW-K,A ↑ - GGT ↑ ALT ↓		- RW-L ↑ - TP, BUN → - cholesterol ↓ - coagulation time ↑	Raju and Devegowda, 2000
OTA-T2 (CM – CM) <i>Chicken</i> (35 d)	2.0 – 3.0				- RW-T ↓		- RW-BF → - Ab ND,IBD ↓		Raju and Devegowda, 2002
OTA-DAS (Pu – Pu) <i>Chicken</i> (19 d)	2.0 – 6.0		- mortality ↓		- RW-G ↑	- BW ↓ - oral lesions ↑ - RW-K ↑ - TP, albumin ↓ - CK ↓	- RW-Pv ↑ - uric acid → - cholesterol ↓	- RW-L ↑ - triglycerides ↑	Kubena <i>et al.</i> , 1994a
OTA-DON (Pu – NCF) <i>Chicken</i> (21 d)	2.0 – 16				- RW-G ↑ - RBC ↑	- BWG ↓ - RW-L,K,Pv ↑ - glucose, TP, albumin ↓	- BUN →	- uric acid ↑ - creatinine → - triglycerides ↑ - cholesterol ↓ - inorganic phosphorus →	Kubena <i>et al.</i> , 1988
OTA-FB (Pu – Pu) <i>Rat</i> (15 d) ³	5.10 ⁻⁶ bw/d – 2.10 ⁻⁴ bw/d	- MDA, PC in liver ↑ - CAT in kidney ↓			- PC in kidney ↑		- MDA in kidney ↑		Domijan <i>et al.</i> , 2007
	5.10 ⁻⁶ bw/d – 0.05 bw/d	- MDA, PC in liver ↑ - MDA in kidney ↑ - CAT in kidney ↓				- PC in kidney ↑			
	0.05 bw/d – 0.05 bw/d	- PC in liver ↑ - PC in kidney ↑ - CAT in kidney ↓				- MDA in liver ↑ - MDA in kidney ↑			
OTA-FB (Pu – CM) <i>Turkey</i> (21 d)	3.0 – 300	- BWG, feed intake ↓ - RW-H ↓ - cholesterol ↓ (P) - creatinine ↑ - AST ↑ (P) - ALT ↑	- uric acid ↑	- BUN ↑	- RW-K ↑	- RW-L,Pc,G ↑ - RW-S ↓ - RBC ↑	- hemoglobin ↑	- triglycerides → - LDH ↑	Kubena <i>et al.</i> , 1997b
OTA-FB (CM – CM) <i>Rabbit</i> (45 d)	2.0 – 10	- TP ↓			- albumin, globulin ↓ - ALP ↑	- creatinine ↑ - ALT, AST, LDH ↑	- glucose ↓		Sivakumar <i>et al.</i> , 2009
OTA-PA (Pu – Pu) <i>Chicken</i>	1.0 bw – 60 bw	- mortality ↑				- BW ↓			Kubena <i>et al.</i> , 1984

(28d) ⁴									
OTA-PA (Pu – Pu) Mouse (21 d)	10 – 40	- RW-S ∇ (P) - mortality ↗				- RW-K ∇ - BW ∇			Shepherd <i>et al.</i> , 1981
OTA-CPA (Pu – Pu) Chicken (21 d)	2.5 – 34	- RW-Pc ↗ - RW-L,K ↗ (P) - TP, cholesterol ∇ (P) - albumin ∇ - CK ↗ (P)			- BW ∇	- uric acid, triglycerides ↗	- RW-Pv ↗		Gentles <i>et al.</i> , 1999
T2-CPA (CM – CM) Chicken (28 d)	1.0 – 10				- % CD4 ⁺ & CD8 ⁺ in thymus ∇ - % CD8 ⁺ in spleen ∇ - lymphocytes stimulation ∇		- % CD4 ⁺ in spleen ∇ - Ab ND ∇		Kamalavenkatesh <i>et al.</i> , 2005
T2-CPA (Pu – Pu) Chicken (21 d)	6.0 – 34	- RW-K ↗ - triglycerides ↗	- RW-L ↗ - cholesterol ↗		- RW-Pc ↗ - albumin ∇	- BW ∇ - mortality, oral lesions ↗ - RW-Pv,G ↗ - RW-BF ∇ - TP ∇ - GGT ∇	- glucose ∇		Kubena <i>et al.</i> , 1994b

1) Mayura *et al.* (1984) : subcutaneously administration during the 20 d-period of pregnancy 2) Siraj *et al.* (1981) : one administration intragastrically and measurements 12 d post administration
3) Domijan *et al.* (2007) : 15 d-treatment with OTA, and treatment with FB for the last 5 days of OTA treatment 4) Kubena *et al.* (1984) : 14 administrations

- **Nature of mycotoxins** : Pu, Purified (>90%); Ex, Extracted from fungal culture material (partially purified); CM, Culture Material; NCF, Naturally Contaminated Feedstuffs; NI, No Indicated in the study

- ↗, ∇, → : evaluation at the end of each experiment, of the effect of the co-contaminated treatment in comparison to the control treatment.

- (P), potentiation

- unless stated, doses are expressed in mg of toxins/kg of feed. In few experiments, doses are expressed in mg of toxins/kg of body weight/day and in mg of toxins/day, and indicated as bw/d and mg/d, respectively.

- **zootechnical parameters** : BW(G), body weight (gain)

- **RW, relative weight of organs** : RW-L, liver; -K, kidney; -G, gizzard; -Pv, proventriculus; -Pc, pancreas; -H, heart; -Lg, lung; -A, adrenals; -S, spleen; -T, thymus; -BF, bursa of Fabricius.

- **biochemical and hematological parameters** : TP, total proteins; BUN, blood urea nitrogen; RBC, red blood cells; WBC, white blood cells; Sa, sphinganine; So, sphingosine; MDA, malondialdehyde; PC, protein carbonyl; CDNB, 1-chloro-2,4-dinitrobenzene; DCNB, 1,2-dichloro-4-nitrobenzene.

- **enzymatic parameters** : ALT, alanine aminotransferase; ALP, alkaline phosphatase; AST, aspartate aminotransferase; GGT, gamma-glutamyltransferase; LDH, lactate dehydrogenase; GDH, glutamate dehydrogenase; CK, creatine kinase; CHL, cholinesterase; CAT, catalase; SOD, superoxide dismutase; GSTP⁺, glutathione-S-transferase placental form positive; EROD, ethoxyresorufin O-dealkylase; PROD, pentoxyresorufin O-depentylase

- **immune parameters** : SMC, spleen mononuclear cells; Ab, antibodies; ND, Newcastle disease; IDB, infectious bursal disease; SRBC, sheep red blood cells; DTH, delayed-type hypersensitivity.

CONCLUSION

In the present review, we analyzed the published data in more than 100 papers, to characterize the interactions between mycotoxins for different parameters. More than half of the published papers investigated the interactions between aflatoxins with other mycotoxins. Although these experiments are relevant in terms of toxicity and natural co-occurrence, we were surprised that only few published papers investigated the interactions between other mycotoxins, especially interaction between toxins from *Fusarium spp.* which are of major concern worldwide.

Overall, the review highlights the complexity of the interactions between mycotoxins. Indeed, even within the same experiment the type of interaction varies according to the parameter measured. Although, most of the studies have shown a synergistic or additive interaction on the adverse effects of the animals performance, the results on other parameters, especially on biochemical compounds, led to different types of interactions, going from synergistic to antagonistic for a same association. Many contributing factors could explain these discrepancies: sensitivity of animal model to mycotoxins, age and sex, nutritional status, as well as the duration and the route of exposure to the toxin. In addition, as reflected in the tables, the levels of mycotoxins used in the experiments may change the type of interaction observed.

Very few studies investigated the effect of mycotoxins, alone or in combination on immune parameters, while in farms vaccination or response to pathogens are common situation. Histopathological analyses provide informations on the organs and cells injuries, but would need to be related with physiological consequences, especially on functions, not often studied (oxidative stress, hepatic drug metabolizing enzymes, intestinal permeability...).

Finally, the number of studies investigating effect of low doses of toxins, representative of field situation is very low. Although the use of moderate to high concentrations of toxins provides information on the type of interaction, these doses are not expected in environmental conditions and are largely over the limit set by regulation/recommendation in different countries. It has been noted in this review that a combination of mycotoxins at low concentration may have negative effects, even though the concentrations of individual mycotoxins are below the concentrations reported to cause negative effects (Domijan *et al.*, 2007). Although not reported in this review, the analysis of interaction with more than two mycotoxins would be also relevant and useful in terms of risk assessment.

Nonetheless, it can be concluded that exposure to a co-contaminated food/feed result in a greater risk to human and animal health. The co-exposure to two toxins led finally to greater total effects in comparison to the total effect of each individual toxin, even in cases categorized less than additive or antagonistic.

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FIGURE

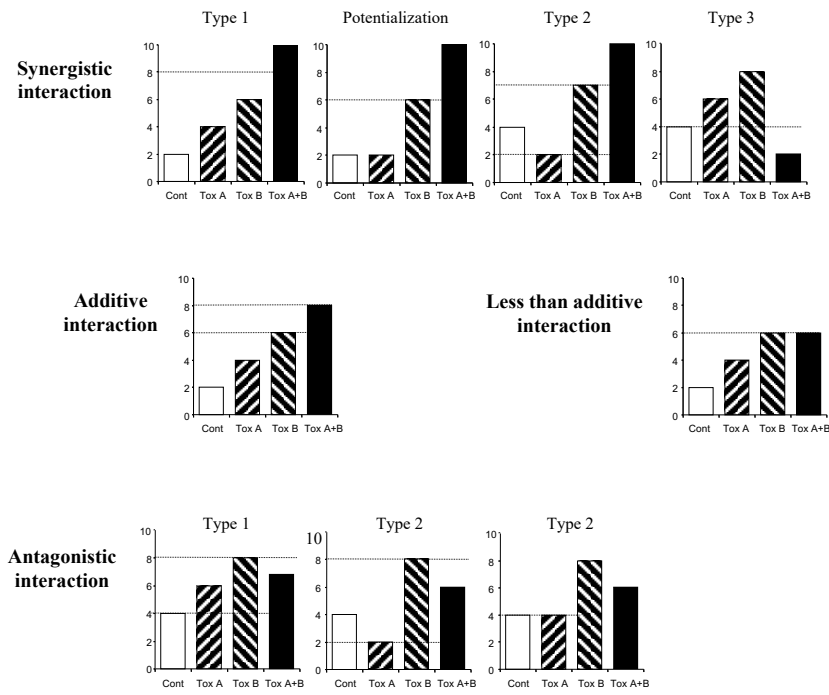


Figure 1 : characterization of the interaction between mycotoxins

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