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Montmorillonite Clay And Lactobacillus Paracasei BEJ01 Mixture Counteracted Immuno-Physiological Alterations From AFB1 In Rats

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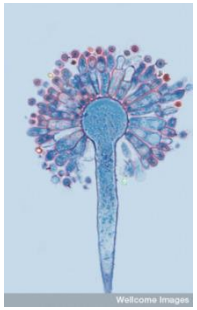
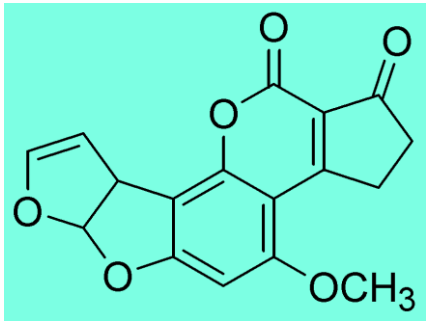
What are Aflatoxins?

- Toxins produced by the fungi *Aspergillus flavus* and *Aspergillus parasiticus*
  - Aflatoxins are secondary fungal metabolites.
  - Aflatoxin types include B1, B2, G1, G2.
  - B1 is most prevalent and toxic aflatoxin.



Microscopic view : spore formation of *Aspergillus*

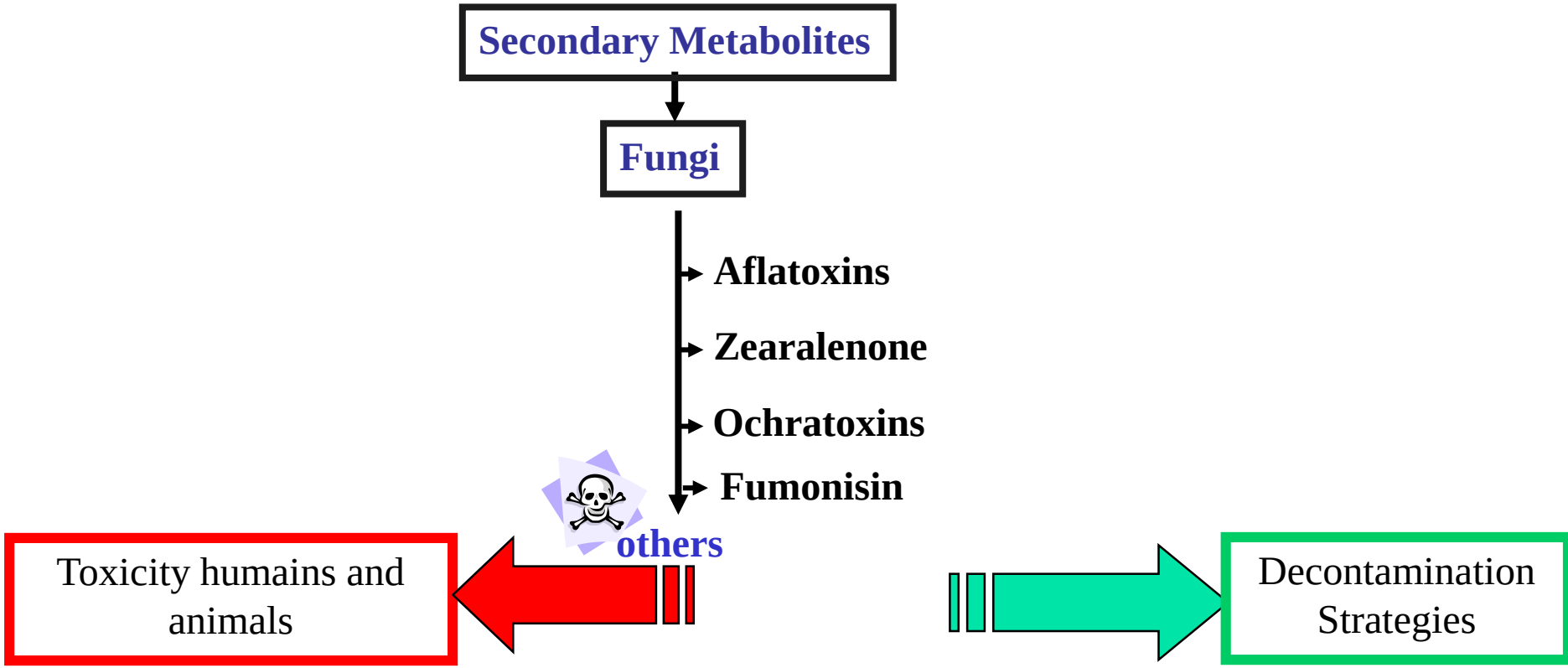


Chemical structure of aflatoxin B1

- Detection:
  - Fluorescence can be used to detect presence of *Aspergillus* on crops
  - Biomarkers are used to detect aflatoxin exposures in humans

Mycotoxins and food Contaminants

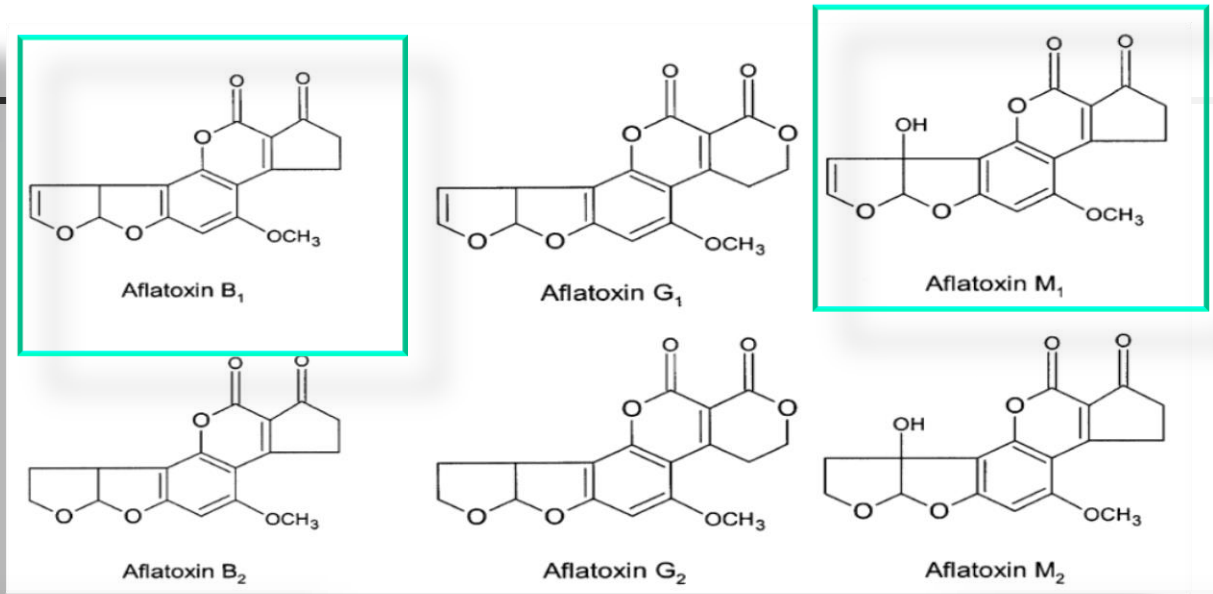
FAO: 25% of food and feed are contaminated with mycotoxins

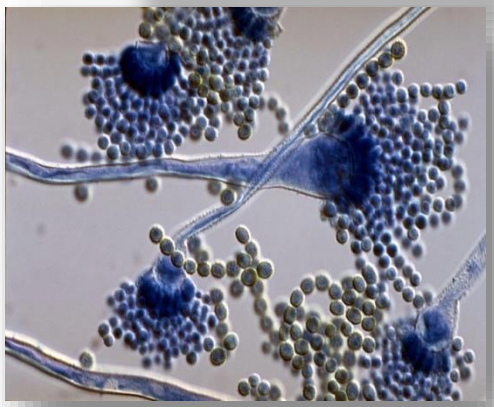


Gibberella ear rot of corn

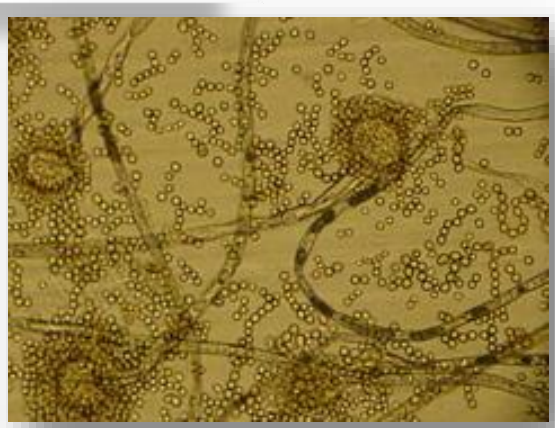
Head blight (Scab) of wheat

Aflatoxins



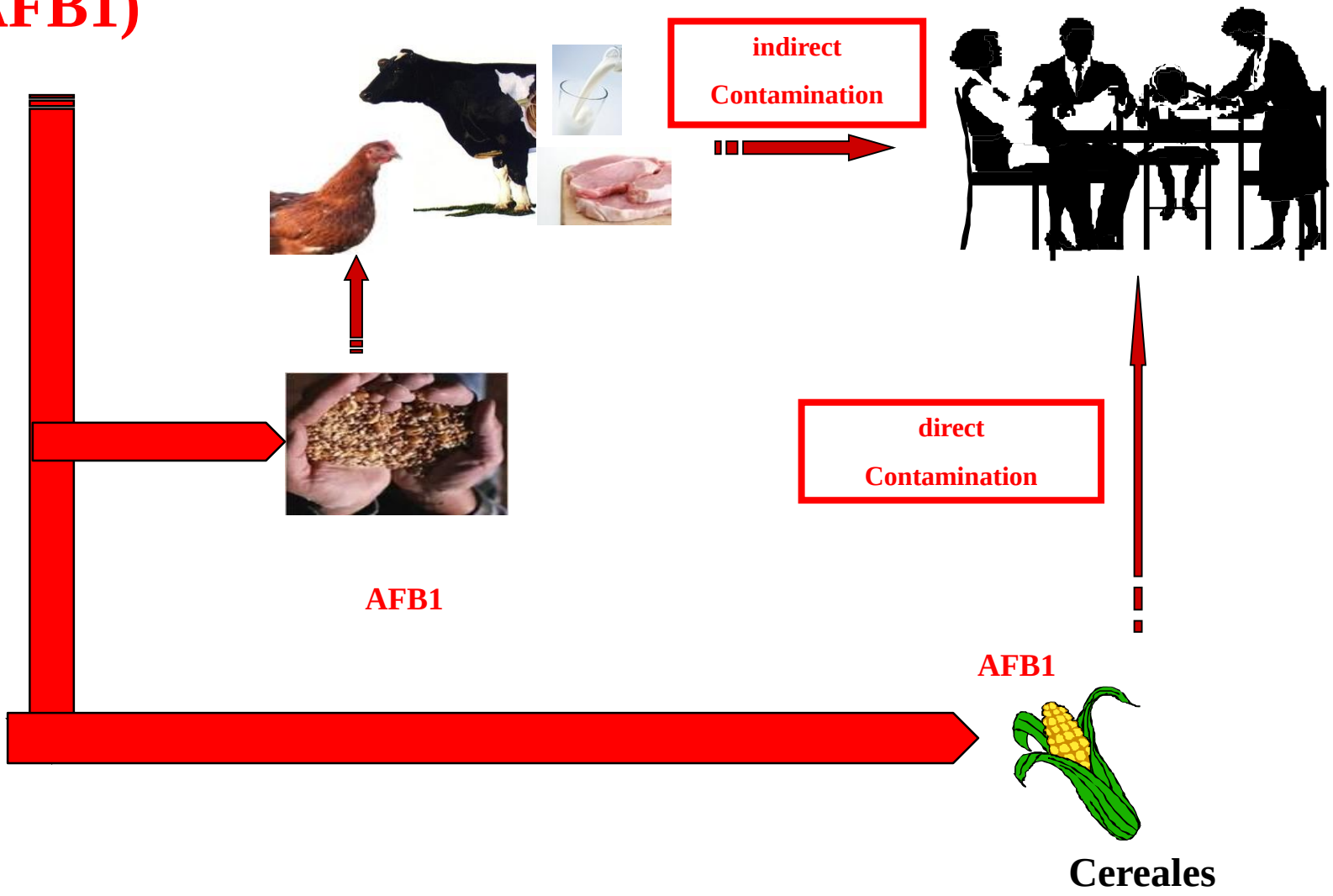


A. Flavus



A. parasiticus

Aflatoxins (AFB1)



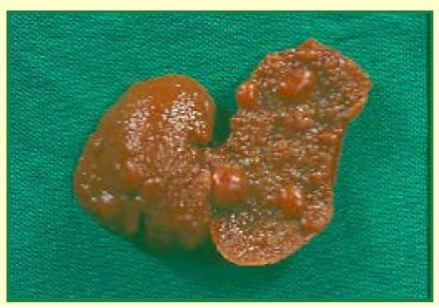
Aflatoxins and Acute Human Health Effects

Acute aflatoxicosis can be **fatal**.

Presenting symptoms are determined by amount of toxin consumed.

Clinical symptoms in humans include:

- Abdominal pain
- Vomiting
- Pulmonary edema
- Liver necrosis



LIVER CANCER IN A RAT

Chronic Aflatoxin Exposure and Human Health

- **Carcinogenicity**
  - Liver cancer is a serious consequence of long-term exposure to aflatoxins.
- Other consequences of chronic exposure include decreased immune and reproductive function.
- Children chronically exposed may experience growth failure.
  - Infants may be exposed through breast milk.
  - The fetus may be exposed during pregnancy if the mother consumes aflatoxins.

No level of aflatoxin exposure is considered safe for humans.

Aflatoxins in Farmed Animals

Poultry

Highly sensitive

Aflatoxin toxicity impairs uptake of essential nutrients as well as causing tissue damage

Ruminants

Ruminants are relatively insensitive; however, aflatoxin exposure can cause growth impairment in young or lactating animals.

Metabolites in milk and related dairy products

Aflatoxin consumed by cows is excreted in milk as the M1 metabolite.

The M1 metabolite also remains present in milk-based products such as cheese and yogurt.

- **Fish**
  - When farmed fish are accidentally fed contaminated grains, large die-offs may occur.

    Rainbow trout are highly sensitive

Animal deaths and reduced productivity from aflatoxin exposure can have significant negative 'economic' impact in addition to the negative health outcomes for those who consume contaminated animal products.

Decontamination strategies of AFB1

Chemical

Organic solvents (chloroform, acetone, hexane and methanol) have been used to extract mycotoxins for agricultural products, but mainly in vegetable oil refining process

Physical

Physically, fungi-contaminated seeds can be removed by hand picking or photoelectric detecting machines. The method would consume time and labor or expensive

Natural compounds .. ??

Decontamination strategies of AFB1

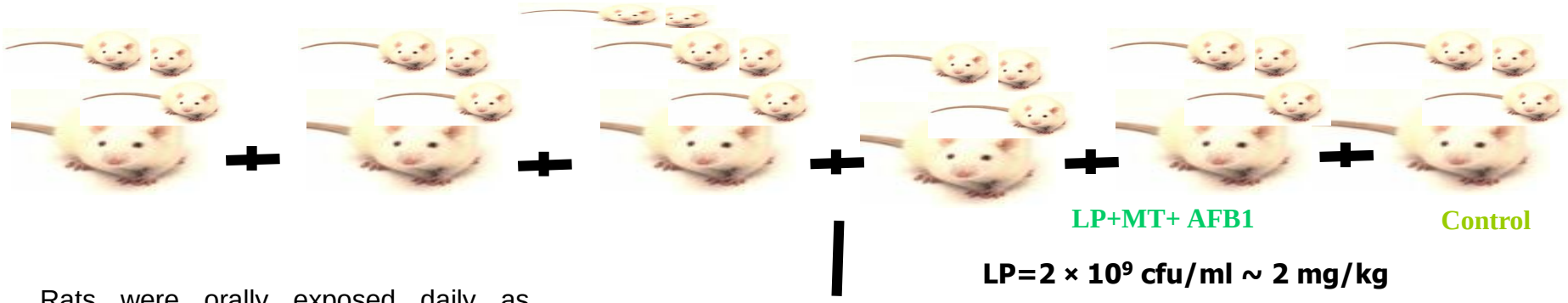
Chemisorbents/Bacteria: Montmorillonite (MT) and Lactobacillus Paracasei BEJ01 (LP)

- The basis of interest in the biological effects of bacteria and Montmorillonite concerns one or more of their physical and chemical properties, such as ion exchange capacity, adsorption and related molecular sieve properties

Materials and methods

MT 0.5 mg/kg bw

AFB1 (100 µg/kg bw)



LP+MT+ AFB1

Control

LP=2 × 10^9 cfu/ml ~ 2 mg/kg

Rats were orally exposed daily as indicated in the Table.

	Week 1	Week 2
Group 1	Control (saline both weeks)	
Group 2	saline	LP+MT
Group 3	saline	AFB_1
Group 4	LP+MT	AFB_1
Group 5	saline	LP+MT + AFB_1
Group 6	AFB_1	LP+MT

General toxicity

AFB1-treated group:

3 rats were died in days 9 and 10 of treatment

→ 30% of mortality

Loss of weight

MT+LP mixture alone or in combination treated groups:

No general toxicity was observed in these groups

Significant gain weight was observed in all groups

Effect of treatments on total WBC and leukocyte counts in rats

Groups	LYMP	WBC	EOS	MONO	PMN
Control	1.25 ± 0.07	8.08 ± 0.14	0.12 ± 0.04	0.46 ± 0.09	6.16 ± 0.08
LP+MT	1.32 ± 0.08	8.10 ± 0.25	0.08 ± 0.05	0.48 ± 0.09	6.11 ± 0.09
AFB_1	*0.69 ± 0.12	*‡12.48 ± 0.40	*0.64 ± 0.07	*‡1.62 ± 0.10	*‡9.81 ± 0.19
LP+MT pre-AFB_1	1.15 ± 0.08	8.10 ± 0.16	0.23 ± 0.06	0.64 ± 0.15	6.04 ± 0.05
AFB_1 with LP+MT	1.23 ± 0.05	8.12 ± 0.17	0.14 ± 0.04	0.55 ± 0.04	6.19 ± 0.09
LP+MT post-AFB_1	1.17 ± 0.04	*‡9.15 ± 0.10	0.24 ± 0.02	*‡0.77 ± 0.17	*‡7.55 ± 0.07

*Value significantly different from control/all other groups ($p < 0.05$); ‡within that subset, values not significantly different from one another.

Effects of treatments on B- and T-lymphocyte subtypes in blood of rats.

	NK($CD56^+$)	B-Lymphocyte	T-lymphocyte subtype		
			$CD3^+$	$CD4^+$	$CD8^+$
Control	1.21 ± 0.41	4.31 ± 1.51	4.51 ± 0.13	3.42 ± 0.21	1.52 ± 0.23
LP+MT	1.27 ± 0.13	4.50 ± 1.32	4.53 ± 0.14	3.34 ± 0.14	1.56 ± 0.25
AFB_1	*0.83 ± 0.09	*2.11 ± 0.13	*2.83 ± 0.15	*2.29 ± 0.13	*0.98 ± 0.22
LP+MT pre-AFB_1	1.22 ± 0.12	4.13 ± 0.55	4.61 ± 0.14	3.41 ± 0.19	1.41 ± 0.08
AFB_1 with LP+MT	1.22 ± 0.17	4.44 ± 0.68	4.61 ± 0.16	3.35 ± 0.16	1.47 ± 0.09
LP+MT post-AFB_1	1.13 ± 0.19	4.37 ± 0.35	4.49 ± 0.14	3.23 ± 0.11	1.35 ± 0.06

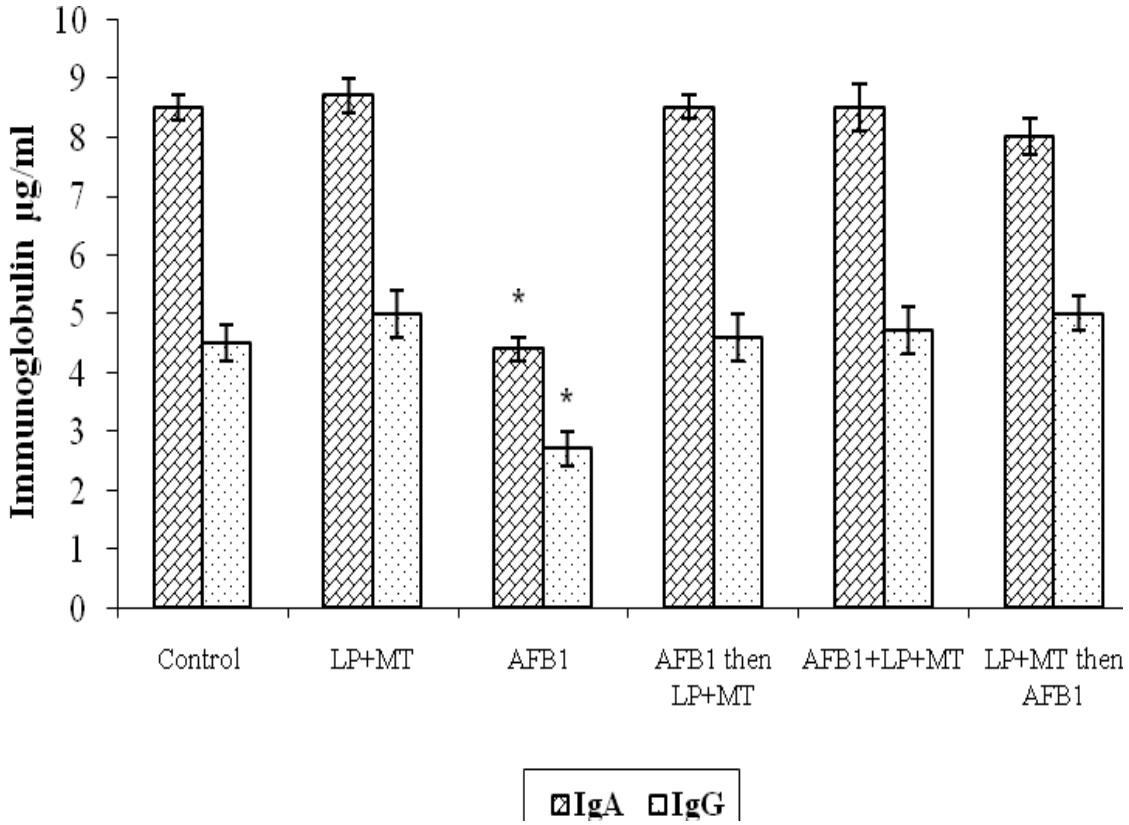
*Value significantly different from control/all other groups ($p < 0.05$).

Effect of treatments on splenic oxidative stress enzymes.

Parameter	Control	LP+MT	AFB_1	AFB_1 pre-LP+MT	AFB_1+LP+MT	LP+MT post-AFB_1
MDA (nmol/mg prot)	61.3 ± 1.5	62.5 ± 2.3	*73.9 ± 5.2	62.7± 6.9	61.9 ± 6.9	61.9 ± 5.9
SOD (U/mg prot)	113.5 ± 7.2	113.9 ± 8.3	*56.2 ± 6.3	119.9 ± 8.6	106.6 ± 6.7	100.9 ± 6.9
GSH (U/mg prot)	126.3 ± 6.9	126.2 ± 6.4	*68.5 ± 6.4	124.9 ± 7.8	121.3 ± 7.9	129.8 ± 5.6
T-AOC (U/mg prot)	28.3 ± 4.6	28.6 ± 2.1	*17.3 ± 3.6	26.3 ± 5.3	27.9 ± 3.5	28.1 ± 6.3

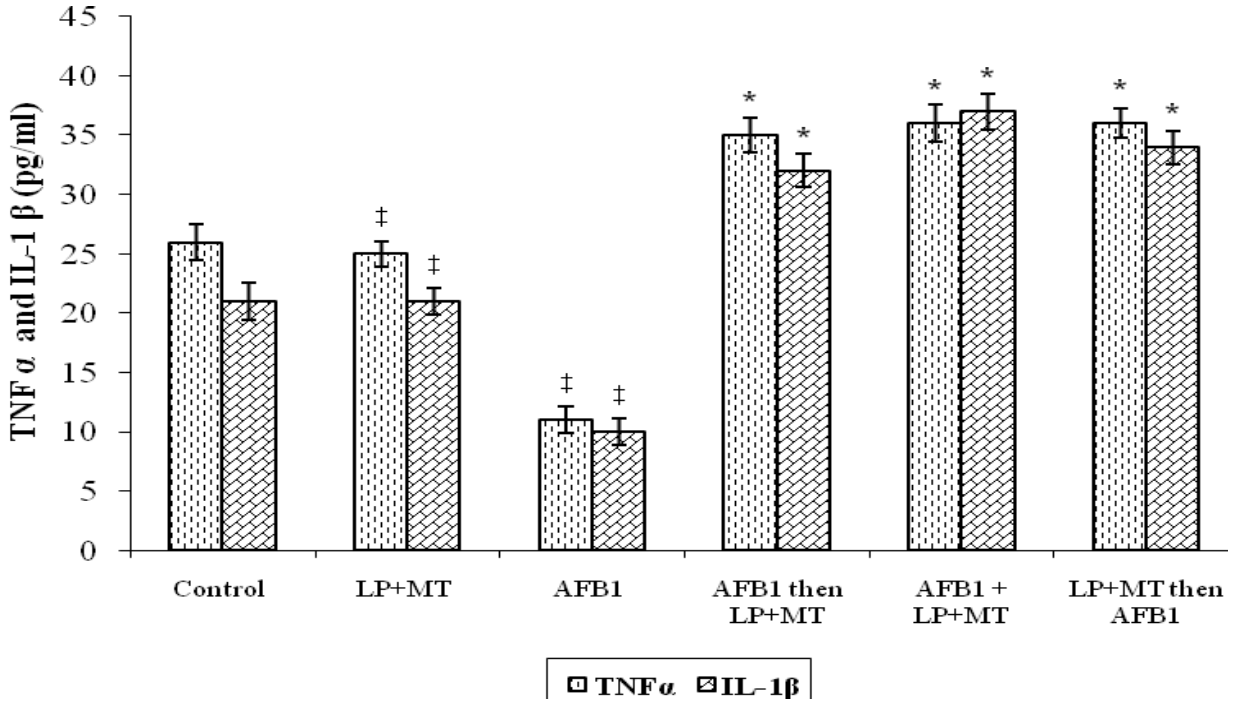
*Value significantly differs from control, LAB alone, or corresponding pre-, co or post-treatment

IgA and IgG levels in rat blood after treatment



*For each given isotype, value significantly different from control and all other treatment group values ($p < 0.05$).

TNFa and IL-1b in plasma from rats after treatment



*For each given isotype, value significantly different from control and all other treatment group values ($p < 0.05$).

Conclusion

- AFB_1 affected the oxidative status in rats, down-regulated B- and T-lymphocyte levels, and negatively impacted on the expression of several key inflammation-regulating cytokines.
- Pre-, co-, or post- treatment using a mixture of *L. paracasei* BEJ01 plus MT imparted protective effects against these AFB_1 immunotoxicities.

Thus, use of a mixture of LP+MT could potentially provide a option for use in protecting against immunotoxicities in many farm animal species that face inadvertent exposure to this class of mycotoxins in their feedstuff.

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RESEARCH ARTICLE

Immuno-physiological alterations from AFB_1 in rats counteracted by treatments with *Lactobacillus paracasei* BEJ01 and montmorillonite clay mixture

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ABSTRACT

High contamination by aflatoxin B_1 (AFB_1) has been detected in Beja province (Tunisia) in many dairy products and animal feed, which has resulted in many tons of cereals and cereals being removed from the market, causing economic loss. While removal represents a means of reducing risk, exposures still occur. Studies have increasingly focused on means of AFB_1 biodegradation/elimination using lactic acid bacteria and clay mineral. In the study here, *Lactobacillus paracasei* BEJ01 (LP) and montmorilonite clay (MT) were used to reduce the physio-/immunotoxicologic disorders that could develop in rats that underwent AFB_1 exposures for a total of 7 consecutive days. The results indicated that rats treated with AFB_1 (80 µg/kg BW) alone had significant decreases in lymphocytes in their blood (including B-lymphocytes, $CD3^+$, $CD4^+$, and $CD8^+$ T-lymphocyte subtypes, and NK cells), immunoglobulins (IgA and IgG) and pro-inflammatory cytokines; these rats also had altered oxidative stress status. In contrast, in rats treated with LP + MT (2×10^9 cfu/ml [$\sim$ 2 mg/kg] + 0.5 mg MT/kg BW) for a total of 7 days before, concurrent with or after AFB_1 treatment, there was a significant blockade/mitigation of each AFB_1-impacted

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Thank you