SELECTED DIETARY APPROACHES TO COUNTERACT AFLATOXIN EXPOSURE ASSOCIATED HEALTH HAZARDS

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The aflatoxins

- **Turkey "X" Disease**
  - Fungal infection by *Aspergillus flavus* and *Aspergillus parasiticus*
  - Primary contamination
    - High energy content foods e.g. grain, nut and soy products
  - Secondary contamination
    - Dairy products, meat & eggs

Available options for solving the problem

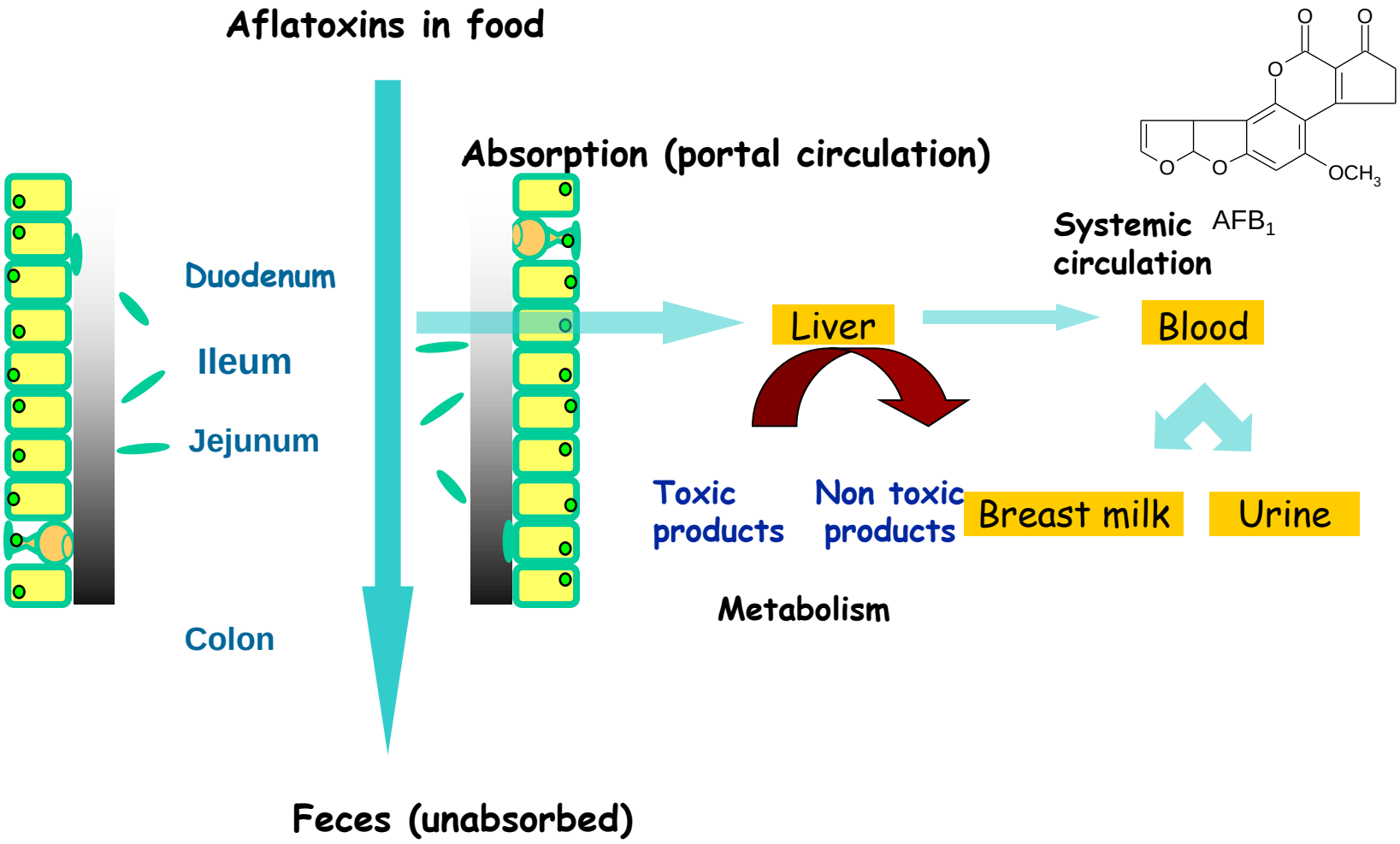
Once food is contaminated with toxins, there are only two options if the food is to be used:

- **the toxin can be removed**
- **the toxin can be degraded into less toxic or non-toxic compounds**

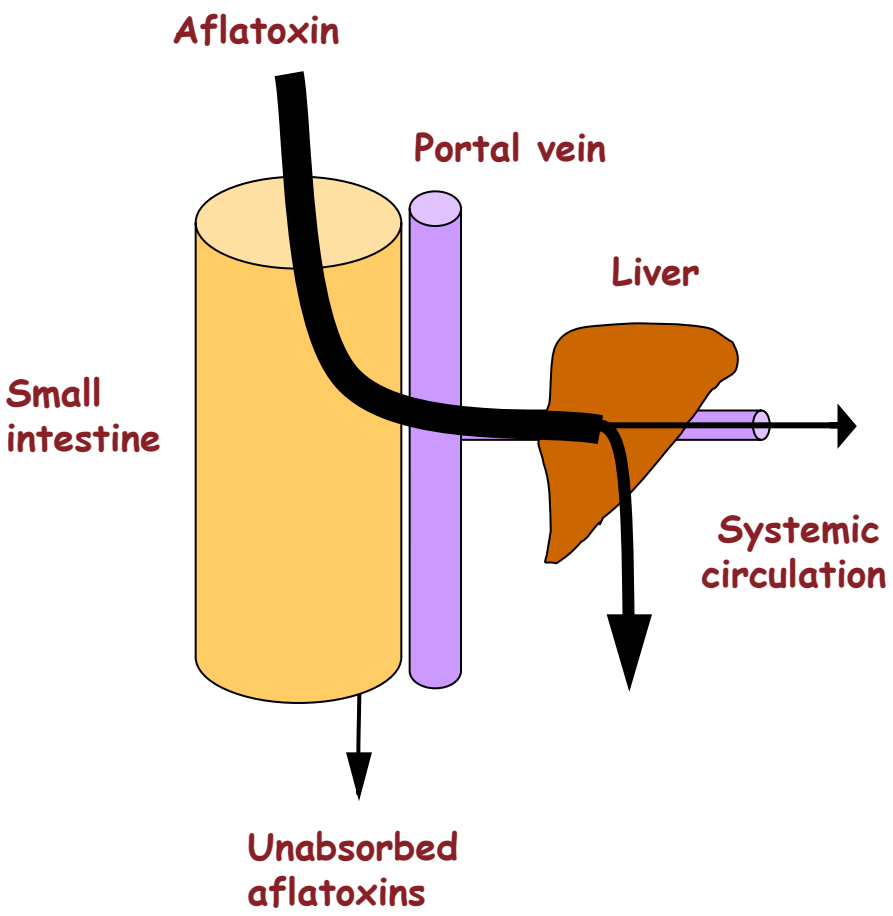
Control measures

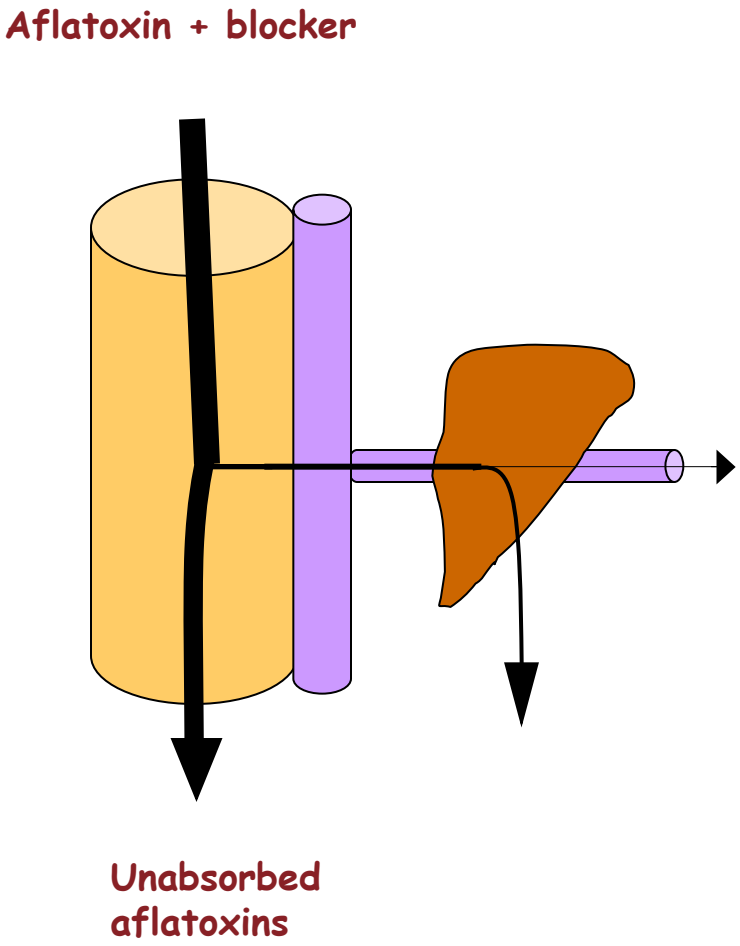
- **Physical control (e.g. UV radiation, electronic sorting)**
  - suitable for very limited products
- **Chemical control (e.g. ammoniation)**
  - health effects are not fully studied
- **Monitoring AF levels and rejection of produce**
  - extremely costly option

Strategies for intervention at individual level



Blocking/reducing absorption of AFB_1 from the small intestine





Requirements for dietary tools of blocking/ reducing aflatoxin absorption in humans

- Part of normal human diet
- Long history of safe use
- Able to bind a range of harmful compounds including aflatoxins
- Binding takes place immediately and is stable under GIT conditions
- No effect on absorption of micro and macro nutrients
- Inexpensive and practical for food enrichments

Microorganisms capable of inhibiting the production and degrading mycotoxins

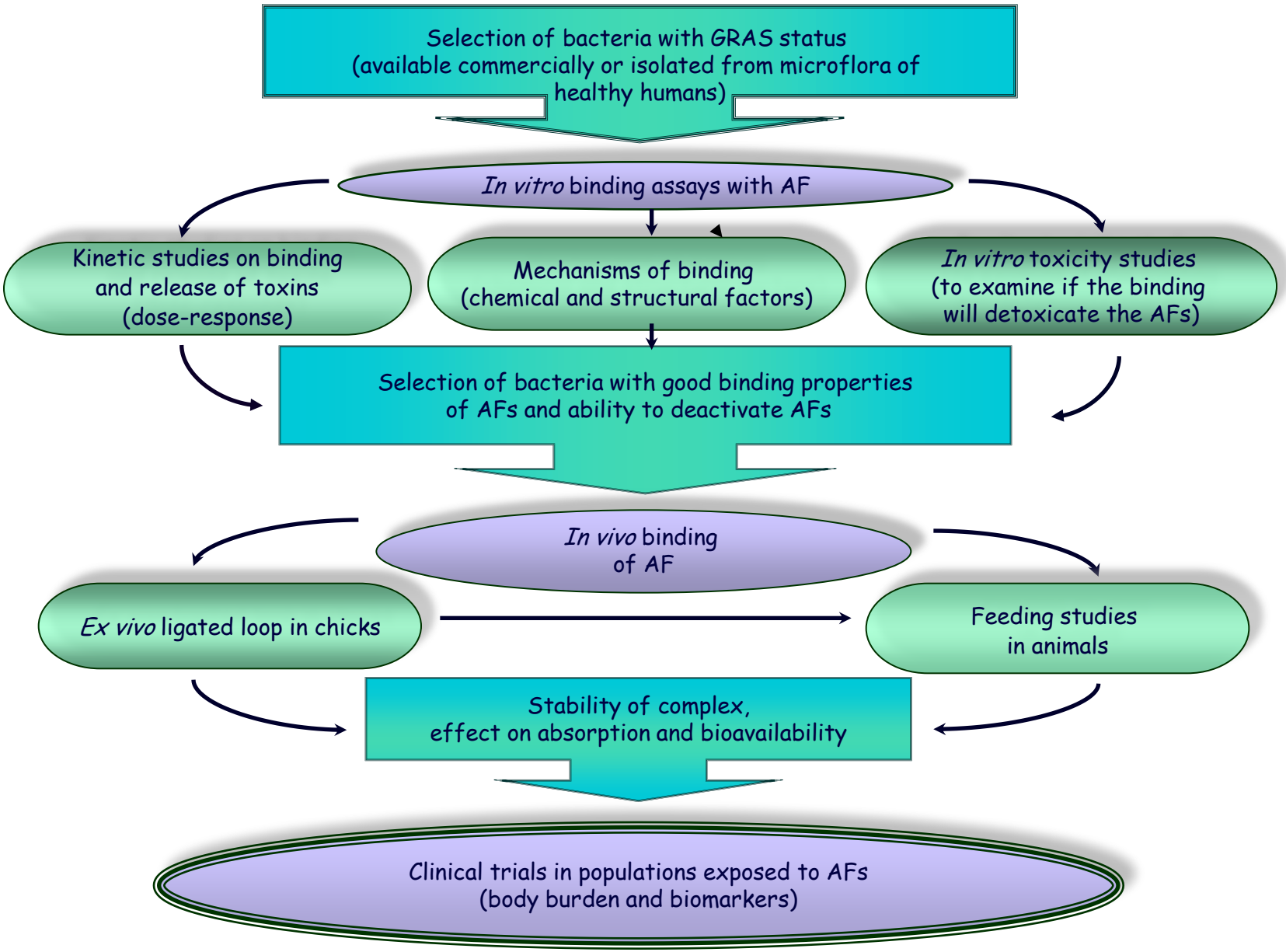
Corynebacterium rubrum
Trichoderma viride
Dactylium denroides
Absidia repens
Rhizopus arrhizus
Rhizopus stolonifer
Aspergillus niger
Mucor ambigus
Mucor griseo-cyanus
Mucor alternans
Rhizopus oryzae
Tetrahymena pyriformis

A range of lactic acid bacteria (LAB), and *animal microflora*

Lactic acid bacteria (LAB)

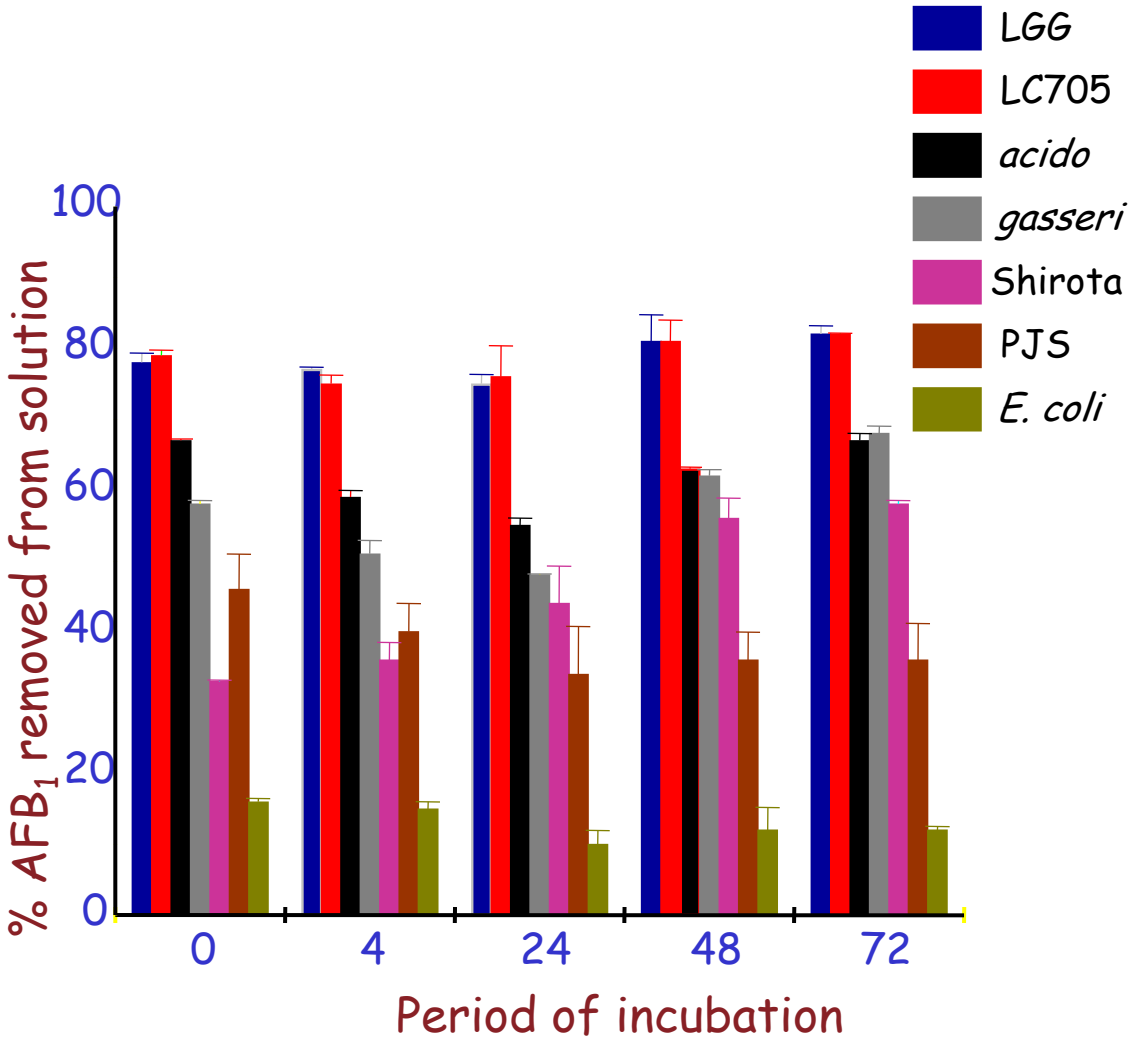
- LAB involved in the production of fermented foods
  - one quarter of our diet
  - characterised by safe history
  - extended shelf life compared to raw materials

- LAB has some health effects
  - growth inhibition of food spoiling bacteria
  - production of antimicrobial compounds
  - probiotic effects as live organisms in food


Selection of bacteria with GRAS status
(available commercially or isolated from microflora of healthy humans)
In vitro binding assays with AF
Kinetic studies on binding and release of toxins (dose-response)
Mechanisms of binding (chemical and structural factors)
In vitro toxicity studies (to examine if the binding will detoxicate the AFs)
Selection of bacteria with good binding properties of AFs and ability to deactivate AFs
In vivo binding of AF
Ex vivo ligated loop in chicks
Feeding studies in animals
Stability of complex, effect on absorption and bioavailability
Clinical trials in populations exposed to AFs (body burden and biomarkers)

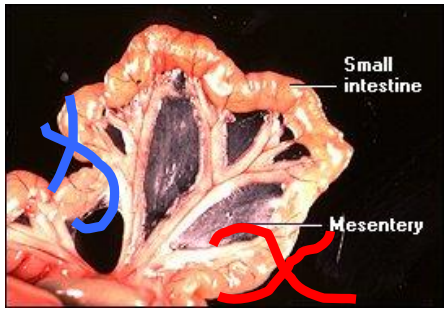
Aflatoxin is bound by probiotic bacteria – *in vitro* evidence

- Certain strains of lactobacilli are capable of binding up to 80% of AFB_1 *in vitro* (El-Nezami *et al*, 1996, 1998a,b,c), Fusarium toxins (El-Nezami *et al*, 2002a,b, 2004), PhIP and Trp-P-1 (Haskard *et al*, 2001)
- AFB_1 is predominantly bound to a carbohydrate moiety on the surface of the bacteria (Haskard *et al*, 2002)
- The complex formed between the bacteria and AFB_1 is stable under different conditions (Haskard *et al*, 2002, Lee *et al*, 2003)



El-Nezami et al, Food Chem Toxicol 1998

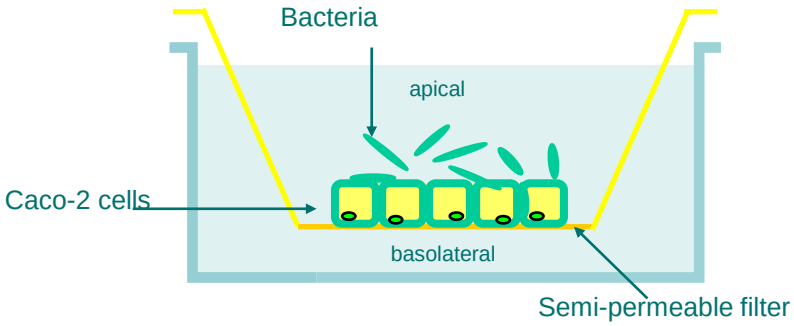
Ex vivo study in chicks

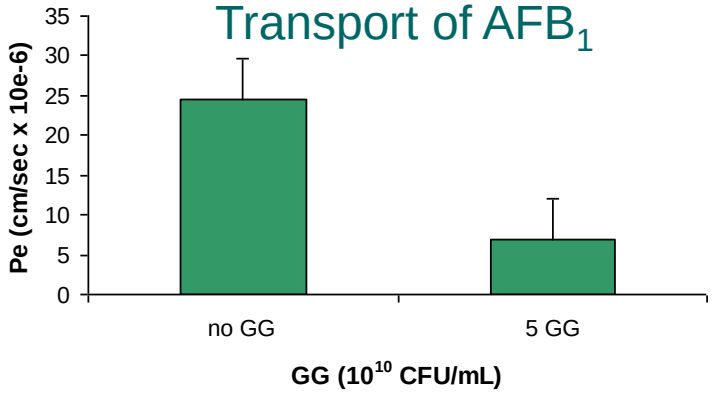


Group	The concentration of AFB_1 ± SD extracted from Duodenal tissue[b]		Soluble fraction[c] of luminal fluid		Insoluble fraction of luminal fluid	
	1min	60 min	1 min	60 min	1 min	60 min
AFB_1 only	0.27 ± 0.09	ND	1.04 ± 0.36	0.05±0.01	ND	ND
LBGG+AFB_1	0.07 ± 0.05	ND	0.48 ± 0.15	ND	0.76±0.04	1.38±0.16
LC705+AFB_1	0.17 ± 0.11	ND	0.58 ± 0.10	0.08 ± 0.06	0.54±0.10	1.07±0.12
PJS+AFB1	0.10 ± 0.05	ND	0.67 ± 0.13	0.13 ± 0.02	0.55±0.11	1.24±0.06

El-Nezami et al. (2000)): Journal of Food Protection. , JGratz S. et al. (2005): Journal of Food Protection.

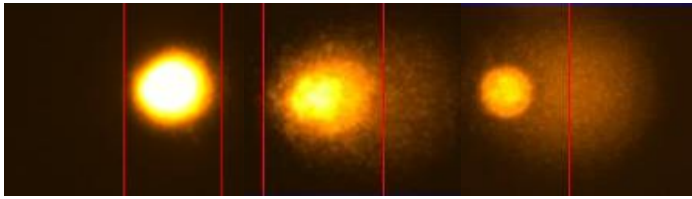
Intestinal AFB_1 transport and toxicity



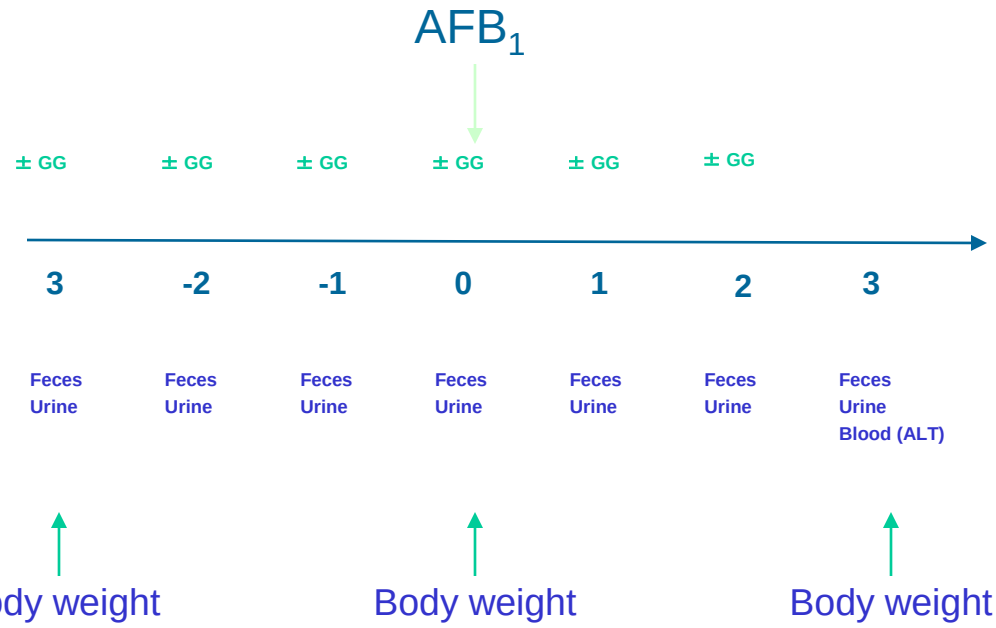


- Transport of AFB_1 through monolayer was reduced by GG
- AFB_1 induced TER (membrane integrity) reduction was attenuated
- AFB_1 induced DNA damage was attenuated

Gratz S., et al. (2007) Applied and Environmental Microbiology.



Single dose study in rats



AFB_1 (1.5 mg/kg bw, single dose on day 0)

GG ($5x10^{10}$CFU, daily for 6 days)

Rat results

GG administration:

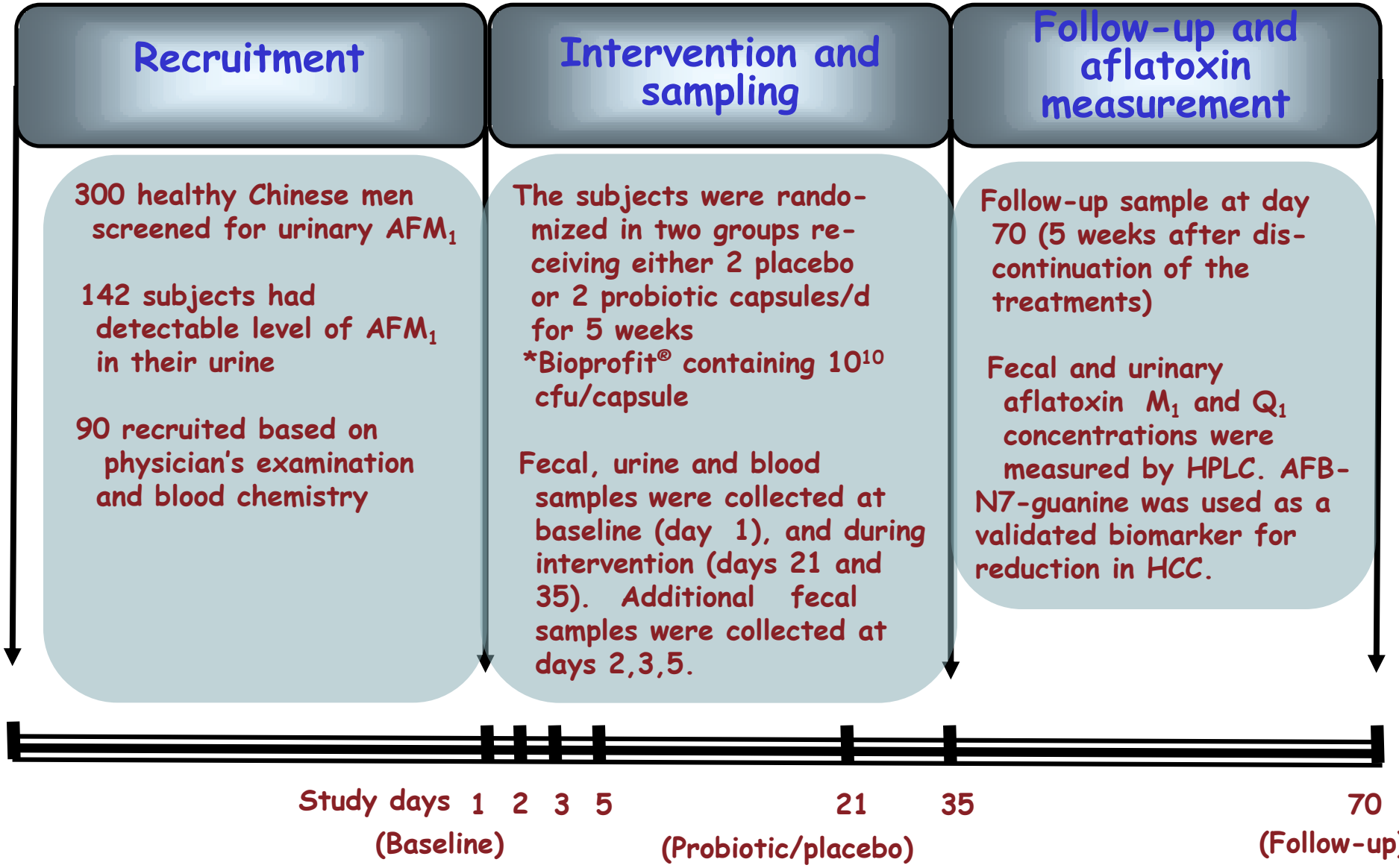
- Increased fecal AFB_1 by 122%
- Increased fecal AFM_1 by 152%
- Decreased plasma AFB_1-albumin by 29%
- Decreased change in liver function (ALT) by 54%
- Prevented body weight loss

Gratz S., et al. (2006): Applied and Environmental Microbiology.

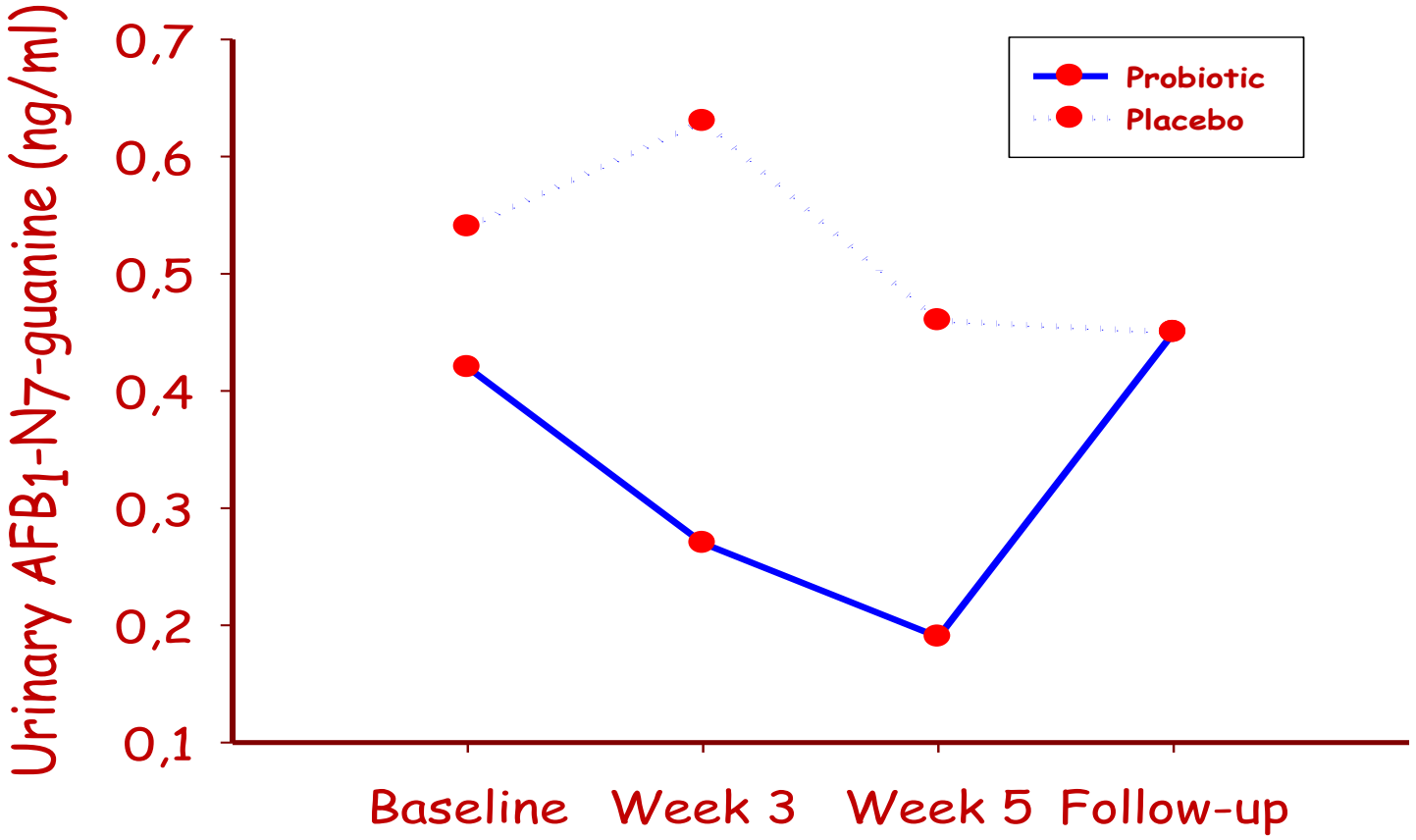
WHY CHINA?

- Primary liver cancer (PLC) is one of the most common cancers in China.
- There more than 250,000 new cases diagnosed yearly with liver cancer in China.
- The mortality rates both in rural and urban areas are 25 and 21 per 100 000, respectively, in the EU 3 per 100,000.
- The main 3 factors for the development of liver cancer are prevalent in China. Aflatoxins are consistent contaminants of the food supply in China, HBV and HCV are endemics in China.
- 500,000,000 individual infected with HBV
  (250,000,000 in China)
- 170,000,000 individuals infected with HCV
  (10,000,000 in China)
- 1,000,000 individuals dies annually because of complication associated with HBV, similar figure also expected for HCV
  (250,000 in China)

Probiotic intervention in China

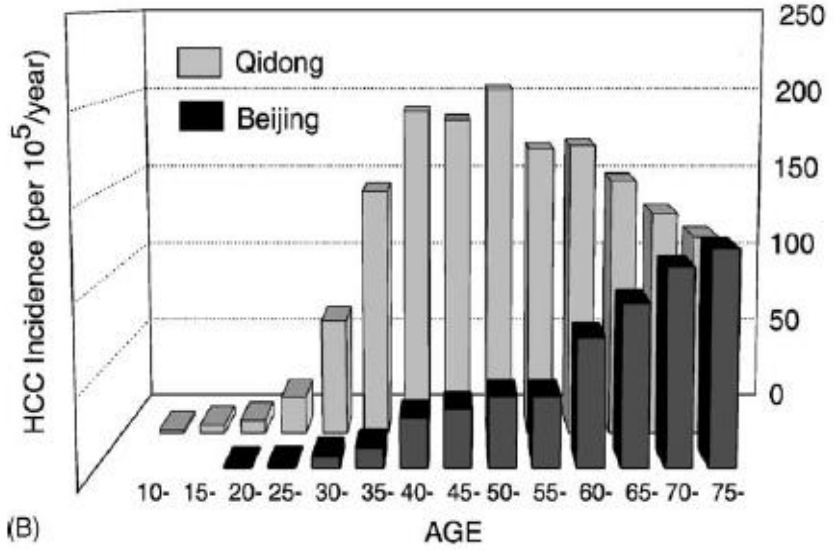


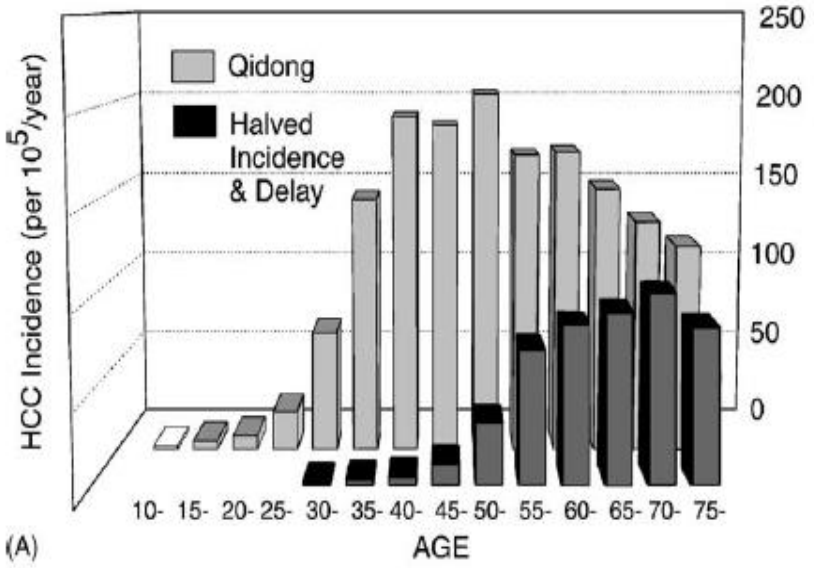
Probiotic supplementation reduces the urinary excretion of AFB_1-N^7-guanine, a biomarker of biologically effective dose of exposure to AFB_1



El-Nezami et al, Am J Clin Nutr, 2006

What our findings mean?





Egner et al, Mutation Res 523-524:209-216, 2003

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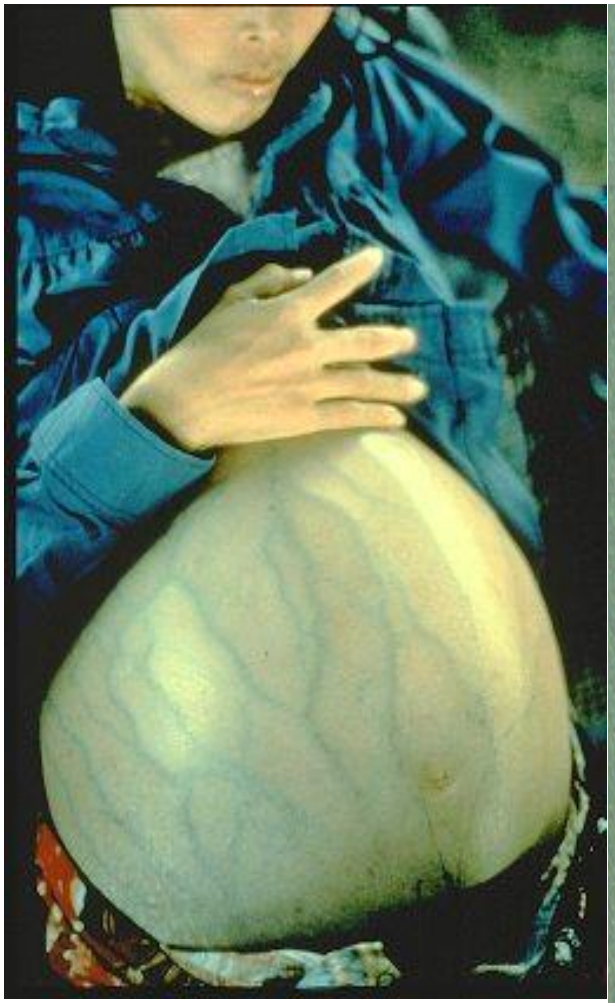

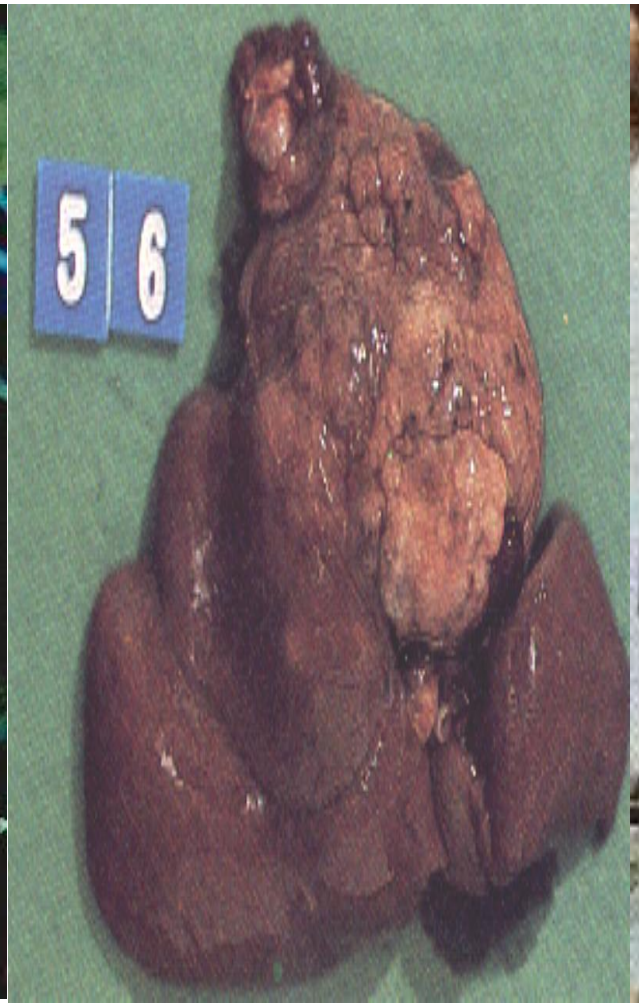
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