EFFECT OF AFLATOXINS ON PIG GROWTH AND THEIR ELIMINATION FROM THE BODY

ALEX C. KONDOS

Summary

The toxic effect of aflatoxins on pigs, their distribution in -and elimination from body tissues were studied in a series of experiments. Results showed that:

1. the response of growing pigs to diets containing 100, 200 or 400 ppb aflatoxin B_1 was in accordance with the increasing concentration of the toxin and in reverse order to the age of the pigs. While the 100 ppb concentration had no detectable effects on the animals, the 200 ppb depressed appetite, growth rate and feed conversion efficiency only in the younger pigs. The highest or 400 ppb treatment was toxic to pigs throughout the experiment.

2. Af Latoxins B_1 and M_1 were present in the liver, heart, muscle, skeletal muscle and kidney 24 hours after treatment but only traces could be detected 48 hours after administration.

3. Metabolism of aflatoxin B_1 was rapid and short term administrations of up to 2 mg B_1/pig resulted in only traces of the toxin and its M_1 metabolite being detectable 24 hours after treatment.

I. INTRODUCTION

Mycotoxins and especially aflatoxins, have been recognised for their adverse effects on animal health and production for many years (Wilson et al. 1967: Wong and Hsieh 1976: Swenson et al. 1977; Stoloff 1982; Blaney et al. 1984: Chauhan et al. 1984; Heathcote 1984: Helferish 1984; Reddy et al. 1984; Wilson et al. 1984).

There are well established differences in the susceptibility of various animal species to the effect of aflatoxins (Goldblatt 1969; Patterson 1973; Roebuck and Wogan 1977) with pigs occupying medium place with regard to sensitivity to these toxins (Jemmali and Murphy 1976; Monegue et al. 1977; Jacobson et al. 1978: Wilson et al. 1984).

Reports on the effect of aflatoxins on pig production are rather confusing and the aim of these investigations was to study the effect of three levels of naturally produced aflatoxins on certain pig production characteristics and the pattern of elimination of aflatoxins from the body tissues of pigs.

IF. EXPERIMENTAL

(a) Toxicity experiment

Large White pigs of mixed sexes and averaging 22 kg body weight were used in groups of seven per treatment. They were kept in single pens and fed one of four experimental diets for 70 days. The four diets had very similar composition (Table 1), with the main variable being the level of aflatoxins calculated by analysis. Their protein content was 18%, lysine 0.9% and were fed to appetite throughout the experiment. There was no evidence of other mycotoxins being present in the diets. The source of aflatoxin was a sample of wheat which contained 505 ppb aflatoxin B_1 (AFB_1) or 805 ppb of total aflatoxin (B_1, B_2, G_1, G_2

Department of Animal Science, Queensland Agricultural College, Lawes. 4343, Queensland, Australia.

Table 1: Composition of experimental diets used in toxicity experiment

Ingredients	CP%	ppb - B_1 0	100	200	400
		% composition of diets			
Wheat - contaminated	13.0	0	20	40	80
Wheat - clean	12.8	79	59	39	0
Soybean	44.0	8	8	8	8
Meat meal	52.8	4	4	4	4
Sunflower meal	32.0	7	7	7	6
Minerals/Vits.* + Salt		1	1	1	1
Limestone		1	1	1	1
CP% in diets		18.0	18.0	18.1	18.0

* The mineral and vitamin mixture supplied the following nutrients in mg kg^{-1} of air-dry diet: zinc, 55; manganese, 24; copper, 7: iron, 88; cobalt, 0.7; selenium, 0.2; vitamins A, 1.2; D, 8.25; E, 0.33; K, 0.66; Niacin, 16; pantothenic acid, 1 mg: riboflavin, 3.3; pyridoxine, 1.1; B_{12}, 0.019; biotin. 0.06; choline-&, 1100.

(b) Tissue distribution and elimination

Large White pigs, weighing approximately 25 kg were individually treated to measure:

1. The distribution of aflatoxins in the tissue of liver, heart, kidney and gluteal muscle. Two groups of 6 pigs each were dosed with 25 or 50 ug B_1/kg Body weight per day for 5 days. Two pigs from each treatment were killed at 3, 24 and 48 hours after the five day aflatoxin administration had been completed.
2. The detoxifying efficiency of the liver and muscle of pigs subjected to two aflatoxin treatments: (a) Each of two pigs was dosed with a total of 4 mg AFB, in 0.8 mg single daily doses for five days. (b) Another two pigs received the same total dose of 4 mg AFB_1 but in two single daily doses of 2 mg each. The pigs were slaughtered 24 hours after the second dose and the liver and muscle were analysed for total AFB_1 and M_1 content.

In each experiment, a pig of similar body weight and with the same background as those used for the aflatoxin observations, but not treated with aflatoxins, were slaughtered and used as controls.

(c) Observations

Inthe growth experiment, growth rate was recorded at 2-weekly intervals and FCE was calculated as being the amount of feed consumed per kg weight gain.

In the distribution and elimination experiments, pigs were killed according tc scheduled times and tissue samples were deep frozen until analysed fcr residual aflatoxins.

Results were tested by analysis of variance.

III. RESULTS AND DISCUSSION

(a) Toxicity Experiment

The toxic effects of the three aflatoxin treatments were assessed over the whole experimental period using as criteria the growth rate, feed conversion efficiency, feed intake and any other signs shown by the aflatoxin treated pigs in relation to the 0 level control group. Results are presented in Table 2 and Figure 1.

Table 2: Cumulative (a) and progressive (b) mean weight gains (kg) per treatment at two weekly intervals, their standard error and significance of differences

Treatments	Weeks from commencement of Experiment				
	2	4	6	8	10
(a) Control	11.2^{a}	20.5^{a}	32.5^{a}	45.3^{a}	59.9^{a}
100 ppb - B_1	10.5^{a}	21.0^{a}	29.1^{a}	42.5^{a}	55.6^{a}
200 ppb - B_1	8.5^{b}	16.0^{b}	25.1^{b}	39.1^{b}	53.5^{a}
400 ppb - B_1	7.0^{b}	13.4^{c}	19.2^{c}	28.5^{c}	40.5^{b}
(Standard Error of $\bar{M}$)	($\pm$ 0.392)	($\pm$0.463)	($\pm$0.910)	($\pm$1.041)	($\pm$1.326)
(b) Control	11.2	9.3	12.0	12.8	14.6
100 ppb - B_1	10.5	10.5	8.1	13.4	13.1
200 ppb - B_1	8.5	7.5	9.1	14.0	14.4
400 ppb - B_1	7.0	6.4	5.8	9.3	12.0

Different superscripts in each column indicate significant differences at 5% or above.

The effect of the aflatoxin treatments on the growth of pigs started becoming apparent as from the end of the first two experimental weeks when the 200 and 400 ppb treatments appeared to exert a significantly depressing ($P < 0.05$) effect on growth. As the experiment progressed, the differences in weight gain between the control and the 400 ppb treatment increased ($P < 0.01$) but by the end of the tenth week. only the 400 ppb treatment remained significantly ($P < 0.01$) be low the other three groups.

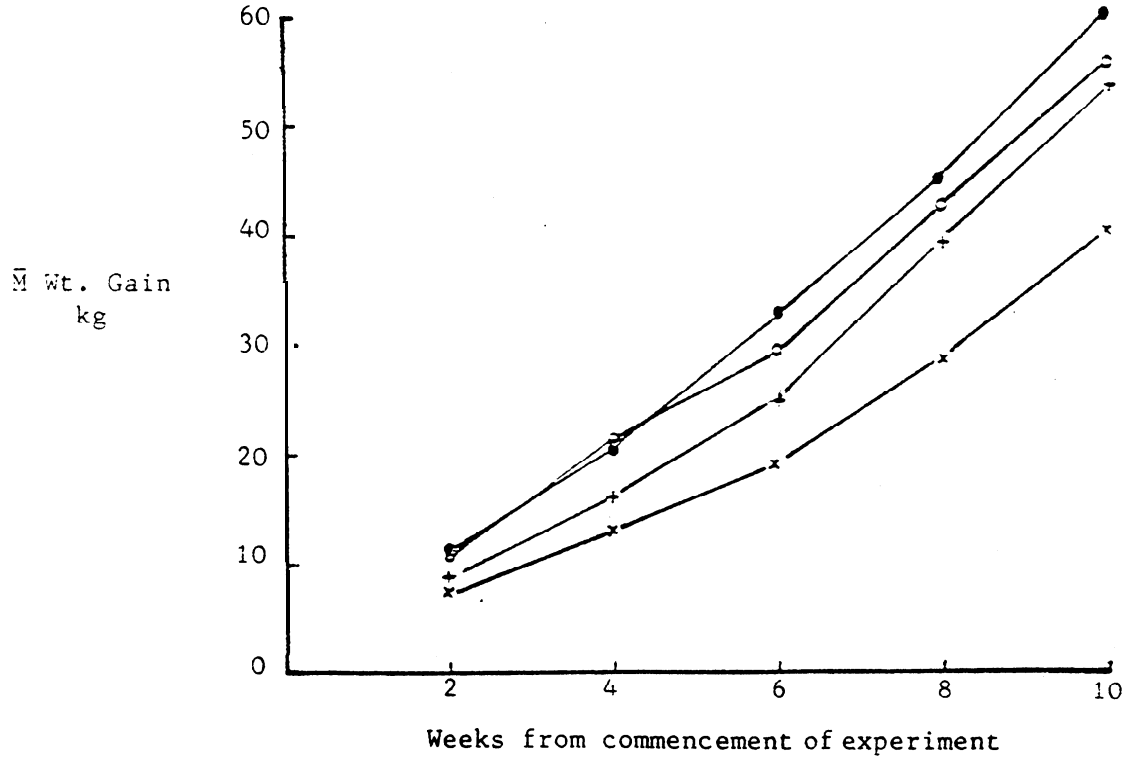

Fig. 1. Mean weight gain (kg) of the control and aflatoxin fed pigs. ●—●, control; ○—○, 100 ppb-B_1; +——+200 ppb-B_1; ×——×, 400 ppb-B_1. Respective slopes are 6.11 kg/pig/wk, 5.85, 5.66 and 4.31.

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From the mean progressive weight gain in each treatment group (Table 2b), it can be deduced that the most severe growth depression was caused by the two highest aflatoxin treatments with the youngest pigs suffering most. The higher degree of susceptibility of younger pigs to aflatoxins has also been reported by Wogan (1968). By the end of the experiment, pigs on the 400 ppb treatment had gained about 33% less weight than the control.

There is conflicting information regarding the lower concentration at which aflatoxins start causing measurable toxic effects on pigs, a rather complex area which can be influenced by a variety of biological and environmental factors. The present results showed that while a 200 ppm concentration of AFB_1 or 319 ppb of total aflatoxin ($B_1 + B_2 + G_1 + G_2$) was toxic to younger pigs, it had no effect on older pigs. On the contrary, 400 ppb AFB_1 or 631 ppb total, affected all ages.

Although Kelly and Booth (1971) reported that 230 ppm B_1 did not cause any toxicity to growing pigs and Monegue et al. (1977) could not detect any appreciable growth depression in pigs fed diets containing 300-500 ppm, on the other hand, Krogh et al. (1973) found that similar levels to those used in our experiments reduced appetite and depressed pig growth.

In most of the published data, aflatoxin toxicity has been associated with the presence of the B_1 aflatoxin only, while the other three aflatoxins (B_2, G_1, G_2) are usually present with B_1 and have also been found to be toxic. Consequently they can contribute to the overall toxicity but to a lesser extent in relation to AFB_1.

The feed intake results (Table 3), in their totality, reflect the growth rate pattern of pigs. The 200 ppb treatment was the lowest level at which an appreciable reduction in feed intake could be detected.

Table 3. Mean feed consumption in kg/pig per treatment and feed conversion efficiency (in brackets)

Treatment	Weeks from beginning of experiment					
	2	4	6	8	10	Total (M)
	Feed consumption - kg					
Control	32.9	27.2	35.1	37.8	43.3	175.4
	(2.92)	(2.96)	(2.93)	(2.95)	(2.90)	(2.93)
100 ppb-B_1	31.1	31.4	23.9	39.5	39.0	164.9
	(2.96)	(2.99)	(2.95)	(2.95)	(2.98)	(2.97)
200 ppb-B_1	25.5	22.2	28.2	42.7	43.2	171.0
	(3.0)	(2.96)	(3.10)	(3.05)	(3.0)	(3.02)
400 ppb-B_1	21.8	20.6	18.3	28.3	36.2	125.2
	(3.11)	(3.23)	(3.15)	(3.05)	(3.02)	(3.11)

Because of the possible effect of aflatoxins on the metabolic functions of the animal, Feed Conversion Efficiency was used to assess whether the growth depression was due to reduced appetite only or to both appetite and metabolic functions. Results give a good indication of the differences in FCE between treatments and especially the consistently inferior mean values amongst the pigs in the 400 ppb group. Although this is only indicative of the possible effects on metabolic functions, assessment of the biological significance of such disturbances in the ability of pigs to efficiently utilize nutrients in the presence of aflatoxins would need a more detailed biochemical study. It is known that the liver, which is the central organ for biochemical transformations, is the target organ for damage by aflatoxins (Polan et al. 1974: Rodricks and Stolloff 1977; Campbell 1977; Stolloff, 1980; Miller et al.1984: Helferish, 1984: Gill et al. 1984; Butler, 1984).

Lijinsky et al. (1970), found that aflatoxin B_1 or one of its metabolites might bind to nucleic acids and proteins resulting in the biological inactivation of these compounds. Similar results, where DNA and RNA had been implicated, have also been reported by Swenson et al. (1973), Gurtoo and Bejba (1974), Ashoor and Chu (1975) and Croy et al. (1978). Their findings showed that aflatoxins can interfere with protein synthesis in the animal, thus reducing the efficiency of protein utilization and consequently growth rate.

According to Patterson (1977), the high hepatotoxic activity of aflatoxin B_1 could be due to an activated hemiacetal form of the toxin which in turn binds to and inactivates certain metabolic liver systems.

From the present results, it appears that the magnitude of toxicity caused by 100-200 ppb AFB_1 was low and this could be due to the high, aflatoxin metabolising capabilities of the pig up to that level of aflatoxin administration (see next experiment). As soon as the level of the toxin was increased to 400 ppb, the tolerance level was exceeded and more severe signs of toxicity become evident.

(b) Distribution and elimination of aflatoxins from body tissues

(i) Low level AFB_1 over five days

Summary of the results on the distribution of AFB_1 in and its elimination from body tissues of pigs is presented in Table 4.

Table 4: Distribution and elimination of aflatoxin B_1 from pig tissues following a two-level treatment

Tissues	Hours after treatment 3 hours	24 hours	48 hours
	ug/kg wet tissue		
25 ug/kg x 5 days	Range	Range	Range
Liver	0.15-0.25	Trace-0.1	Trace
Heart	0.10-0.20	Trace	Trace-0
Kidney	Trace-0.26	Trace	Trace-0
Muscle	Trace-0.20	Trace	Trace-0
50 ug/kg x 5 days			
Liver	0.22-0.40	Trace-0.10	Trace
Heart	0.12-0.34	0-0.14	Trace
Kidney	0.10-0.38	Trace-0.10	Trace-0
Muscle	Trace-0.3	Trace	Trace

The results indicate that, following administration of AFB_1 at the rate of 25 or 50 ug/kg body weight, very small amounts remained in the muscle of pigs slaughtered 24 hours after treatment and only traces at 48 hours.

The hydroxylated M_1 metabolite of AFB_1 was found to be present at very low levels only in the liver and kidney tissue of pigs slaughtered 3 hours after removal of the toxin from the feed. No measurable amount of M_1 was found in pigs slaughtered 24 hours after the aflatoxin contaminated feed was withdrawn.

The rapid metabolism and excretion of AFB_1 from the body may be one of the factors contributing to the appreciable degree of tolerance of the pig to the toxic effects of aflatoxins. No aflatoxin was detected in the control pigs.

The practical importance of the results is that if an aflatoxin contaminated feed had been fed to pigs, the carcass should be considered safe for human consumption about 48 hours after such feed was removed.

(ii) High level AFB_1 over 2 or 5 days

Results from these experiments are presented in Table 5.

Table 5. Level of B_1 and M_1 in the liver and muscle of Pigs (ug/kg) fed the same total quantity of B_1 in two different daily rates

Tissues	24 hours after end of treatment	
800 ug x 5 = 4 mg	B_1	M_1
Liver	0.1 ppb	Trace - 0.1 ppb
Muscle	0.1 ppb	Trace - 0
2000 ug x 2 = 4 mg		
Liver	0.20- 0.1	0.1 ppb
Muscle	0.15- 0.1	Trace - 0.1

Despite the low residual level of B_1 and M_1 in the liver and muscle tissue 24 hours after end of treatment, there is a distinct difference between the two treatments in the absolute concentration of residual aflatoxins in each tissue.

Comparing these resultswith those in the previous experiment (Table 4), it is interesting to note that pigs have the ability to rapidly metabolise AFB_1 at least within the dose rates used in these experiments. Although the simplicity of these experiments does not allow for any extrapolation of the results for toxicological interpretation of the findings, there are indications that as the dose rate of AFB, increases, there is a reduction in the rate at which detoxification takes place.

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