THE UNIVERSITY OF MANITOBA

EFFECT OF MOLDY MYCOTOXIN-FREE BARLEY AND OCHRATOXIN A CONTAMINATED BARLEY-BASED DIETS ON YOUNG GROWING PIGS

BY

CORNELIA CHRISTINA LIPPOLD

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CORNELIA CHRISTINA LIPPOLD

A thesis submitted to the Faculty of Graduate Studies of the University of Manitoba in partial fulfillment of the requirements of the degree of

MASTER OF SCIENCE

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ABSTRACT

Ochratoxin A (OA), a mycotoxin produced by the Aspergillus or Penicillium mold species, may develop on grain and feeds in storage. OA contamination of feeds is common in Europe and western Canada and it is toxic to growing pigs. Consumption of OA contaminated feeds by pigs can result in degenerative kidney changes described as mycotoxic nephropathy.

Molded feeds may reduce feed utilization in domestic animals independent of mycotoxin contamination. Therefore moldy mycotoxin-free (MB) barley-based starter pig diets were tested in the first experiment. In the second and third experiment barley-based starter and grower pig diets contaminated with mycelia containing OA were evaluated. Mycelia containing OA was used to simulate moldy OA contaminated barley. In the last experiment the hypothesis of developing a tolerance towards OA by feeding increasing concentrations of OA was tested. The residual effects of MB and OA were tested in the first and third experiment, respectively.

The inclusion of 50% MB in the diet decreased feed intake ($P<0.05$). Average daily gain was impaired ($P<0.05$) after the administration of 25% and 50% MB, but feed efficiency was not significantly impaired. Amino acid digestibilities were decreased ($P<0.05$) with 50% MB in the diet. Administering 2.7 mg kg^{-1} OA in the diet resulted in reduced ($P<0.05$) feed intake. Average daily gain was depressed after feeding 2.5 and 2.7 mg kg^{-1} dietary OA, but feed efficiency was not significantly impaired. Succeeding a MB or OA contaminated diet with a control diet resulted in a return to normal performance.

Serum creatinine and serum total protein, indicators of kidney

impairment, were increased ($P<0.05$) after the administration of 0.5 mg kg^{-1} OA in the diet, but levels returned to normal values after the experimental diets had been replaced by control diets. Serum parameters indicating extrarenal damage were changed after the administration of 2.5 or 2.7 mg kg^{-1} OA in the diet. These were increased serum globulin ($P<0.05$) and serum calcium ($P<0.05$) concentrations, decreased serum glucose ($P<0.05$) and γ-glutamyl transpeptidase activities ($P<0.05$) concentrations.

Pigs did not adapt ($P>0.05$) to 2.5 mg kg^{-1} OA after they had been conditioned with 0.5 mg kg^{-1} OA.

Based on the results of these experiments, less than 25% MB or 0.5 mg kg^{-1} OA in the feed may be administered for a short period of time. Additional research is needed on the effect of FDM or mycelia containing OA on serum parameters, because, compared to other studies, more serum parameters were affected.

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FOREWORD

This thesis is written in manuscript style. Both manuscripts will be published in the Canadian Journal of Animal Science.

The authors of the manuscripts are: C.C. Lippold, S.C. Stothers, A.A. Frohlich, R.R. Marquardt and G.H. Crow, Department of Animal Science, University of Manitoba, Winnipeg.

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INTRODUCTION

Wet, warm storage harvest conditions favor the growth of post-harvest fungi such as the Penicillium and Aspergillus species. Toxigenic fungal strains may evolve over time and consequently, ochratoxin A (OA), a secondary metabolite toxic to monogastric animals, may be produced. OA has been found on various grains and feeds in Europe (Krogh 1978) and western Canada (Prior 1981) since its first identification on naturally contaminated corn in the United States (Shotwell et al. 1969). Climatic conditions in parts of Manitoba are favorable for mold production and OA is the most common toxic contaminant (Andrews et al. 1981). Levels as high as 27.0 mg kg^{-1} OA have been observed in feed samples in Saskatchewan (Scott et al. 1972), but the normal range observed for OA is 0.01 mg kg^{-1} OA (Sinha et al. 1986) to 6.0 mg kg^{-1} OA (Andrews et al. 1981).

Feeding an OA contaminated diet to market pigs results in OA residues in blood and tissues and may be a human health hazard (Petkova-Bocharova and Castegnaro 1985). OA has been detected in blood from slaughter pigs in Poland (Golinski et al. 1984, 1985), Sweden (Hult et al. 1984), West-Germany (Bauer et al. 1984) and western Canada (Marquardt et al. 1987). Its presence indicates that OA contamination in feed may be a problem. Therefore, research on the effect of OA on growing pigs was carried out at the Department of Animal Science, University of Manitoba, Canada.

In the Prairie Provinces moldy grain contaminated with the Penicillium and Aspergillus species is usually not contaminated with mycotoxins (Sinha et al. 1986), but research data in this area were limited. Therefore the effects of moldy mycotoxin-free barley were

studied first. The main objective was to determine if the mycotoxin-free mold had a detrimental effect on pig performance, crude protein and amino acid digestibility. Another objective was to clarify if the mold had any residual effects on pig performance.

Secondly, the effects of OA contaminated barley-based diets on pigs were tested. The hypothesis of developing a tolerance to OA was tested on pig performance, serum OA and biochemical blood parameters. Thus, in this part of the study, low and high levels of OA were tested as well as low level followed by high level feeding of OA. As in the first part of the study another goal was to determine if OA had any residual effects on pig performance, serum OA and biochemical parameters.

LITERATURE REVIEW

1. Field and storage toxins - brief overview

Mycotoxins are secondary metabolites of filamentous fungi which are detrimental to animals and man when ingested (Scott 1985a). Table 1 summarizes the toxins of major concern in Canada.

In the field, plant debris, soil and infected crops form the growth substrate for mold spores. The dispersion of spores takes place through air movement, insects or birds. During the floret stage or even up to the soft dough stage grain kernels are receptive to germination of spores. Temperatures of 15-35°C and frequent rain fall are essential for inducing fungal growth (Seaman 1985, Hunter and Sutton 1985). With the predominance of toxigenic mold strains mycotoxins may be produced. Field mycotoxins once produced can persist during storage (Abramson and Mills 1985).

Development of post-harvest fungi is promoted by storing grain at high moisture and temperature or by development of moist pockets in the grain bin through moisture leakage into the bin (Trenholm et al. 1985). Primary fungal metabolites include fatty acids, fatty alcohols and CO_2. Fungal odors are caused by the fungal volatiles 1-octen-3-ol, 1-octanol, 3-octanone and 3-methyl-1-butanol (Abramson et al. 1980, 1983). Toxigenic fungal strains may produce secondary metabolites, mycotoxins, such as aflatoxins, sterigmatocystin or ochratoxins (Abramson and Mills 1985). Figure 1 summarizes the steps to mycotoxin production in storage.

2. Ochratoxin A - Chemical structure, occurrence and mode of action

Ochratoxins are secondary metabolites of several fungal <u>Aspergillus</u> and <u>Penicillium</u> sp. or subspecies (ssp.). Ochratoxin A (OA), the most

Table 1. Field and storage toxins of major concern in Canada

Fungi	Mycotoxins produced	Occurrence
Field fungi		
Fusarium	zearalenone	Corn and other grains
	Trichothecenes deoxynivalenol (DON, vomitoxin)	Winter wheat, corn
	T2 toxin	Grains, hay
Claviceps	ergot alkaloids	Grains, grasses, weeds more common on open-pollinators like rye and grasses, seldom on self-pollinators like wheat, barley, oats except in wet weather at anthesis
Storage fungi		
Aspergillus	aflatoxins	Peanuts, cottonseed corn, hay, flaxseed
	sterigmatocystin	Wheat, barley
	ochratoxin A	Barley, wheat other grains,
Penicillium	ochratoxin A	forage, hay

From: Scott et al. (1985).

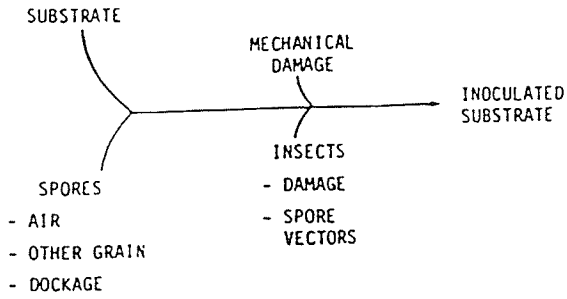

Fig. 1a.

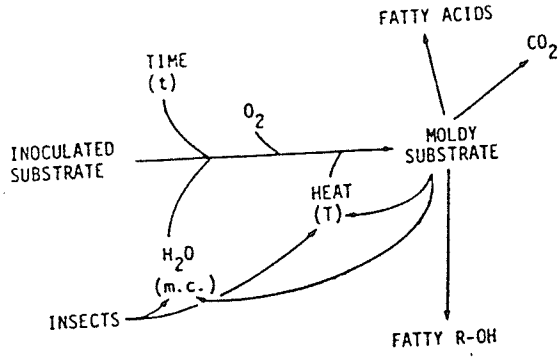

Fig. 1b.

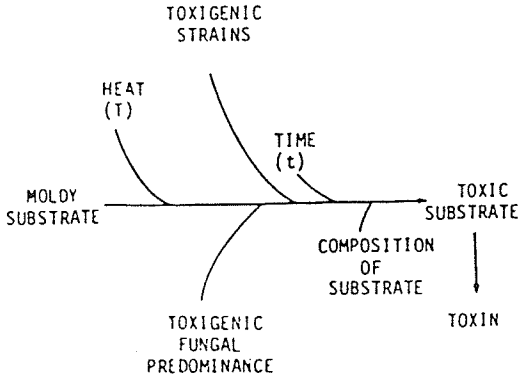

Fig. 1c.

Fig.1. Conditions of mycotoxin production in post-harvest storage.

From: Abramson and Mills (1985)

toxic compound (Chu et al. 1972), is a dihydro-isocoumarin derivative linked through its 7-carboxygroup to L-β-phenylalanine as depicted in Figure 2a. It was first isolated by South-African researchers from cultures of Aspergillus ochraceus Wilhelm, (Van der Merwe et al. 1965), a fungus that grows well under subtropical or tropical conditions. Penicillium verrucosum var. cyclopium (Westling) Samson, Stolk and Hadlok and Penicillium viridicatum Westling have been identified since as OA producers in moderate climates (Abramson et al. 1982, Abramson and Mills 1985, Abramson et al. 1985, Krogh 1978). Other Penicillium and Aspergillus sp. or ssp. are also OA producers (Mills and Abramson 1982, Scott 1985b, Chu 1974).

OA prevents protein synthesis by competing with phenylalanine for the phenylalanine-t-RNA synthetase (Roschenthaler et al. 1984). In an in-vivo study, Creppy et al. (1984) measured the percentage of protein inhibition in mice liver and kidney. They observed that the kidney was more susceptible to protein inhibition than the liver. Recent research suggests the possibility of tumor formation in liver and kidney by OA metabolites produced in the liver (Kane et al. 1986).

Acid hydrolysis of the amide bond between phenylalanine and the dihydroisocoumarin moiety produces Ochratoxin α (Oα), (Figure 2b) a nontoxic derivative. Feeding 1 mg Oα to day-old chicks produced no ill-effects, however, feeding 0.2 mg OA to day-old chicks resulted in a 90% death rate (Chu et al. 1972).

OA is hydrolysed in the body although the site of detoxification and the amount hydrolysed is controversial. Patterson et al. (1976) suggested that OA was detoxified in the intestine before being absorbed. In laboratory studies, homogenates of rat pancreas, duodenum and ileum

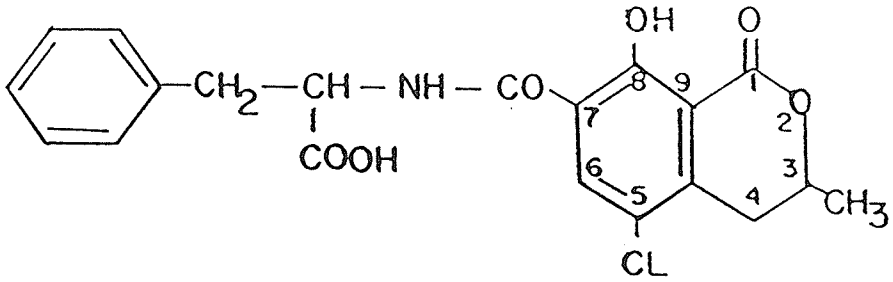

Fig. 2a. Ochratoxin A

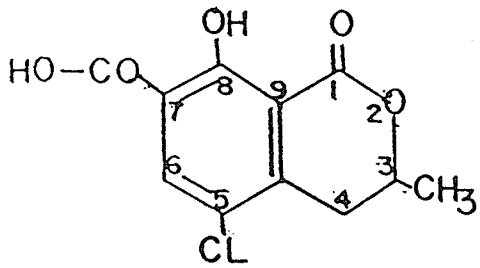

Fig. 2b. Ochratoxin α

Fig. 2. Chemical structure of ochratoxin A and ochratoxin α.

Adapted from Chu (1974)

hydrolysed OA, but Oα could not be detected in rat liver or kidney homogenates (Suzuki et al. 1977). Moroi et al. (1985) tested mice liver homogenates for OA hydrolysation, but again no Oα could be found. Creppy et al. (1984), however, hypothesized that the liver might be a site of detoxification, because a smaller degree of protein inhibition had been measured in the liver of mice compared to that observed in the kidney of mice. Moroi et al. (1985) suggested that hydrolysis was taking place in the intestine. Oα and part of OA are mostly excreted through feces (Suzuki et al. 1977, Patterson et al. 1976).

3. Experiments with growing pigs

3.1 Performance

3.1.1 Administration of molds or moldy feed

Fungal proteins have been tested for nutritive value in animal feeds (Reade and Gregory 1975, Nielsen and Gregrory 1978). Fungi of the Aspergillus, Fusarium and Trichoderma species (sp.) were incorporated into a semi-synthetic diet as the only source of protein and were compared to casein-based diets when fed to grower pigs (Smith et al. 1975). Apparent N-digestibility and apparent net protein utilization (NPU) of the fungal protein were 27-51% and 15-30% lower than the casein, respectively. Fungal proteins were deficient in the S-containing amino acids (AA), methionine and cystine. The addition of methionine improved the NPU of the fungal protein, however, fungal protein was still of lower biological value than the casein. Santos and Gomez (1983) observed that fungal protein supplemented with methionine resulted in a lower feed intake, but similar performance to soybean meal diets with pigs in the 15-90 kg liveweight range. Forster et al. (1986) substituting half of the protein of a starter pig diet with the fungal

mass of Chaetomium cellulolyticum Chahal and Hawksworth for a three week period did not report any adverse effects on performance.

While molds grown and prepared under controlled conditions may be a good source of protein, mold infested grains in uncontrolled conditions are of concern to the farmer, since the nutritional properties and toxic potential of the grain is undefined. Jones et al. (1955) replaced normal oats and/or wheat with moldy oats and/or wheat containing Aspergillus, Penicillium and other species in starter and grower/finisher pig diets and tested feeding value, palatability and digestibility. In general, palatability was not decreased as could be seen in average daily feed consumption, average daily gain (ADG) and feed to gain ratios. As the moldy oats was incorporated into the diets at a low level (25 or 36%), the authors concluded that differences in feeding value might have been masked by the other ingredients of the experimental diets. Lower bushel weights of the moldy oats corresponded to higher crude fibre values. In addition the organic matter, crude protein, nitrogen-free extract (NFE) and gross energy digestibility were decreased.

3.1.2 Administration of ochratoxin A contaminated feed

Danish research indicates that less than 0.2 mg kg^{-1} OA in naturally contaminated feed will not induce adverse effects in swine during the 20-50 kg growth period (Madsen et al. 1982a). Madsen et al. (1982a) restricted-fed market pigs when administering naturally OA contaminated feed. In the 20-50 kg liveweight range, when feeding at concentrations of 1.4 mg kg^{-1} OA, the daily feed allowance had to be reduced by up to 26% whereas the administration of 1.9 mg kg^{-1} OA in the 50-90 kg liveweight range caused a reduction of the daily feed allowance

by only 4%. Krogh et al. (1974) did not observe any change in daily feed intake after feeding 4.0 mg kg^{-1} OA from 20-90 kg. However, from 20-50 kg and 50-70 kg the daily feed intakes (feeding to scale) were 6-20% and 2-10% lower than the ones described by Madsen et al. (1982a), respectively.

In another experiment, an OA contaminated diet (1.4 mg kg^{-1} OA) was fed to starter pigs (20 kg liveweight) for six weeks followed by a toxin-free diet for the 35-70 kg liveweight range. Performance in this latter period was comparable to control values indicating that no residual effect of the toxin was present (Madsen et al. 1982a).

Concentrations of 0.2, 1.0 and 4.0 mg kg^{-1} OA in feed administered in the 20-90 kg range resulted in reduced weight gains and feed conversion. The adverse effects were especially noticeable at the highest concentration (Krogh et al. 1974). Madsen et al. (1982a) studied the influence of OA during the early (25-50 kg) and later (50-90 kg) growth period. Exposures to 1.4 and 1.9 mg kg^{-1} OA in the feed during the 25-50 and 50-90 kg liveweight range, respectively, reduced weight gains.

Madsen et al. (1982b) compared the potency of crystalline OA to OA present in naturally contaminated barley and found that performance was depressed to a greater degree by the naturally contaminated barley. They suggested that fungal odors were partly responsible for the reduced feed intake and that other unknown factors were acting synergistically with OA.

3.2 Ochratoxin A residues in blood and tissues

OA residues in blood are positively correlated to OA concentrations

in tissues and feed (Mortensen et al. 1983). Both tissue (Mortensen et al. 1983) and feed (Hult et al. 1980) concentrations of OA have been calculated from OA concentrations in blood. A plateau level of OA in blood and tissues is reached after having fed the same dietary OA concentration for two to three weeks (Madsen et al. 1982a, Hult et al. 1979, Mortensen et al. 1983).

Most of the OA present in blood is bound to albumin (Rutquist et al. 1978) and yet unidentified low molecular weight plasma constituents. The low molecular weight species are able to pass the glomerular membrane and accumulate in the kidney (Stojkovic et al. 1984), where the highest concentration of OA in tissues is found (Krogh et al. 1974, 1976, 1979, Madsen et al. 1982a, Mortensen et al. 1983). There is no agreement on the distribution of OA in the other tissues. No adequate explanation for the differences can be given, but one explanation might be the sampling of muscle and fat from different parts of the carcass. OA was detected in decreasing order in muscle, liver, fat (Madsen et al. 1982a, Mortensen et al. 1983), liver, muscle, fat (Krogh et al. 1976, 1979) or liver, fat, muscle (Krogh et al. 1974).

Naturally contaminated barley when added to a standard diet caused higher residues in blood and tissues than crystalline OA (Madsen et al. 1982b). The authors suggested that other unknown factors in moldy barley might act synergistically with OA and therefore increase the amount deposited.

When a standard diet replaces an OA contaminated diet, OA is eliminated at an exponential rate from blood and tissues. The half-life was calculated to be six days for OA in blood (Mortensen et al. 1983) and 3.3, 4.3, 4.5 days for OA in muscle, liver and kidney, respectively

(Krogh et al. 1976). No half-life for OA in fat was determined by Krogh et al. (1976).

3.3 Biochemical parameters in blood and urine

Plasma, serum and urine samples have been tested for abnormal levels of metabolites after the administration of OA in the feed.

Increased total plasma protein was considered to be a sign of dehydration (Szczech et al. 1973) as observed in renal disease. Reduced glomerular filtration rates were indicated by elevated creatinine (Krogh et al. 1974, 1979, Tapia and Seawright 1984) and blood urea nitrogen (BUN) levels (Szczech et al. 1973). Both creatinine and BUN are normally filtered by the glomerulus and excreted through the urine (Coles 1974). Glutamic oxaloacetic transaminase (GOT) was the only enzyme to show increased activities in the serum after the ingestion of very high OA concentrations (Szczech et al. 1973). Since GOT is present in many tissues, it is an indicator of hepatic and muscular cell damage only in conjunction with other enzymes (Kramer 1980).

Renal damage was confirmed through the presence of protein and glucose in the urine (Krogh et al. 1974, 1979, Szczech et al. 1973, Zimmermann et al. 1979, Tapia and Seawright 1984). An increase in albumin and proteins with similar molecular weight in the urine suggested a higher permeability of the glomerulus to macromolecules (Krogh et al. 1974). Zimmermann et al. (1979) also found blood in the urine resulting from leakage of hemoglobin or myoglobin through the glomeruli or from extravasation of red blood cells into the urogenital tract (Finco 1980). Increased activities of leucine aminopeptidase (LAP), (Krogh et al. 1974), lactate dehydrogenase (LDH), isocitrate dehydrogenase (ICDH) and GOT (Szczech et al. 1973, Zimmermann et al.

1979) in the urine implied kidney damage, because all of these enzymes were considered to be of renal origin.

3.4 Nephrotoxicity

Mycotoxic porcine nephropathy is caused by chronic exposure to diets containing less than 10 mg kg^{-1} OA (Elling 1977, Krogh 1980). Extrarenal effects, such as a fatty infiltration of the liver, enteritis, necrosis of the lymphoid tissue and leucocytosis have been observed at higher concentrations of OA (Szczech et al. 1973) which are rarely found in naturally contaminated feed.

3.4.1 Renal function

Polydipsia and polyuria are visible clinical symptoms of mycotoxic nephropathy. In a water deprivation test, a standard test for measuring kidney function, the observed low specific gravity of the urine will not increase suggesting that pigs lost the ability to concentrate urine. Reduced glomerular filtration rates and tubular absorption were indicated by a reduced para-aminohippuric acid (PAH) and inulin (C_{IN}) clearance. Decreased maximal tubular excretion (Tm_{PAH}) and a lower ratio of Tm_{PAH}/C_{IN} occurred which are signs of reduced kidney transport capacity (Krogh et al. 1974).

3.4.2 Renal structures

Gross pathological changes produced by OA included enlarged kidneys pale in color (Elling and Moller 1973, Elling 1977). Microscopical lesions are confined to the cortex. In the proximal tubules a reduction in height of the brush border and desquamation of the epithelial cells are followed by tubular atrophy coupled with a thickening of the basement membrane. Additional changes in advanced cases include focal interstitial fibrosis, degenerative changes of the glomeruli and cysts

glomeruli and cysts containing dilated lymphatics (Elling 1977). Administering 0.2 mg kg^{-1} OA in feed in the 20-90 kg liveweight range produced microscopical changes in four out of nine pigs without any gross pathological changes (Krogh et al. 1974). After two weeks of exposure to 1.4 mg kg^{-1} OA in feed, four out of ten starter pigs exhibited clinical changes whereas only one animal showed histological damage. The impairment progressed with the length of exposure and all the animals displayed signs of mycotoxic nephropathy after six weeks (Elling 1983). Elling (1983) also found that renal lesions were more advanced in younger pigs and were not reversible when a toxin-free diet succeeded the experimental diet (2.3 mg kg^{-1} OA in the feed) for the 70-90 kg liveweight growth phase.

4. Prevention and treatment of moldy grain

4.1 Prevention - use of mold inhibitors

Research has been directed towards the use of preservatives as mold inhibitors for high moisture grain. Propionic acid and ammonia when added at a concentration of 1 and 2% respectively, to yellow corn reduced mold growth with propionic acid being more efficient at preventing toxin production (Vandegraft et al. 1975). A N-oxime ether had the same or superior mold inhibitory properties as propionic acid, but it was heat labile which would probably make it unsuitable for pelleting (Burditt and Hamilton 1983).

In a yeast extract-sucrose (YES) medium at pH 4.4, potassium sorbate was most efficient in preventing mold growth and toxin production, followed by sodium propionate, methyl paraben and sodium bisulfite. At pH 5.5, methyl paraben and potassium sorbate prevented mold growth and toxin production more efficiently than sodium

propionate. Sodium bisulfite was not suitable at the higher pH range (Tong and Draughon 1985).

Sorbic acid added at 0.1% to a high moisture sorghum diet or a reconstituted sorghum diet was effective in preventing mold growth when stored aerobically for seven days. No adverse effects of the sorbic acid on pig performance were noted (Crenshaw et al. 1986).

The concentrations and effectiveness of preservatives are dependent on the moisture content of the grain (Burditt and Hamilton 1983). Repeated applications may be necessary to insure an efficient treatment (Vandegraft et al. 1975).

4.2 Treatment - detoxification of Ochratoxin A contaminated barley

Three methods of detoxification (ammoniation, heating and addition of NaOH or autoclaving) were evaluated by Madsen et al. (1983) in feeding and residue studies. Although all three methods reduced the amounts of OA in the feed substantially, pig performance did not improve as anticipated and tissue OA residues were higher than expected. Since extra soybean meal had been added to the autoclaved barley diets to account for lost amino acids during the autoclaving process, the authors concluded that OA metabolites were responsible for the lower performance. They also reasoned that partial reconversion into OA must take place under the acid conditions of the pig's stomach in order to explain the persisting OA residues in kidney and tissues.

MANUSCRIPT I: EFFECT OF MOLDY MYCOTOXIN-FREE BARLEY ON YOUNG GROWING PIGS

ABSTRACT

Pig performance, crude protein and amino acid (AA) digestibilities were studied for 28 days (part 1) when 25 or 50% standard barley of a starter pig diet was replaced by 25 or 50% mycotoxin-free moldy barley (MB). Performance was also recorded when a control grower pig diet was fed to each treatment group in part 2. Increasing the concentration of MB in the diet decreased feed consumption ($P<0.05$) and gain ($P<0.05$), but not feed efficiency. The crude protein digestibility was not affected ($P>0.05$), but a higher concentration of MB in the diet decreased AA digestibilities ($P<0.05$). In part 2, growth returned to normal when pigs were fed the mold-free grower diet. The evidence indicated that increasing dietary concentrations of MB resulted in a progressive impairment of performance when fed to young pigs. This impairment was reversible.

Key words: Growing pigs, moldy barley, performance, digestibility

INTRODUCTION

Wet harvest conditions in the fall or during a spring harvest, favor growth of storage fungi. Such conditions are a problem in western Canada, especially in the Peace River Valley in Alberta or in the Red River Region in Manitoba. Even without the development of mycotoxins the acceptability and nutritional value of the feed may be affected.

Since information of moldy toxin-free grains on pig performance is limited, a study was initiated to determine if barley contaminated with Aspergillus flavus Link and Penicillium verrucosum var. cyclopium (Westling) Samson, Stolk and Hadlok, had a detrimental effect on pig performance, crude protein and amino acid digestibilities. A second objective was to evaluate performance when a control diet had replaced the experimental diets.

MATERIALS AND METHODS

Animals and Diets

Thirty-six (36) pigs (Managra, York and Managra x York crosses) were divided into two weight groups (16.1 ± 0.2 kg and 18.5 ± 0.2 kg starting weight ±SD) and were allotted to six pens with three barrows and three gilts per pen. Each pen measured 2.6 x 2.9 m and no bedding material was used on the concrete floors.

Control barley-soybean meal starter and grower pig diets were formulated to meet or exceed NRC (1979) requirements. Moldy Bedford barley had been collected from a storage bin in Elkhorn, Manitoba in the spring of 1985 and was found to be contaminated with the storage fungi Aspergillus flavus Link and Pencillium verrucosum var. cyclopium (Westling) Samson, Stolk and Hadlok. Experimental diets were mixed in a vertical Marion Mixer (Rapids Machinery Co., Marion, Iowa, USA) (Table

Table 2. Starter pig diets and their performance in Part 1 of the experiment

	Clean Bedford barley	Moldy Bedford barley	Experimental diets† 0% MB* T_1	25% MB T_2	50% MB T_3	SE
Proximate analysis (%)						
Dry matter	88.5	87.9	88.8	88.6	88.6	-
Ash	2.6	2.8	5.9	6.1	6.0	-
Crude protein	11.6	14.5	15.8	17.1	18.3	-
Crude fat	1.5	1.0	1.5	1.5	1.4	-
Acid detergent fibre (ADF)	7.1	9.4	6.7	7.3	7.9	-
Chromic oxide	-	-	0.4	0.3	0.4	-
Amino acid analysis (%)						
Lysine	0.43	0.40	0.82	0.84	0.79	-
Methionine	0.28	0.27	0.30	0.34	0.34	-
Cystine	0.31	0.30	0.34	0.38	0.38	-
Threonine	0.36	0.45	0.60	0.62	0.61	-
Performance						
Average daily feed consumption/pen (kg)	-	-	8.63a	7.27ab	5.33b	0.21
Average daily gain (kg)	-	-	0.59a	0.43b	0.28c	0.02
Feed:gain ratio	-	-	2.44	3.01	3.61	0.16
Digestibility (%)§						
Lysine	-	-	46.6ab	52.8a	41.7b	2.3
Methionine	-	-	54.7ab	56.7a	45.4b	2.6
Cystine	-	-	77.3a	76.0a	69.9b	1.2
Threonine	-	-	51.1ab	56.6a	48.2b	2.1

†Composition of starter diet was: 82% barley, 15% soybean meal (47%), 1% dicalcium phosphate, 1% limestone, 0.25% trace mineralized salt, 0.25% medicated vitamin and mineral mix and 0.5% chromic oxide. Trace mineralized salt provided the following nutrients per kg of diet: 2.4 g sodium chloride, 0.25 mg iodine, 0.1 mg cobalt, 4 mg iron, 0.83 mg copper, 3 mg manganese, 10 mg zinc.

Medicated vitamin and mineral mix provided the following nutrients per kg of diet: 2200 IU vitamin A, 330 IU vitamin D, 12 µg vitamin B_{12}, 123 mg zinc oxide (88.6 mg zinc min.), 2.5 g mecadox.

*% MB = Percent moldy barley of the diet; T = treatment, SE = standard error.

§Overall crude protein digestibility ±SE = 61.8% ± 1.0.

a-c Means with different letters within a row differ significantly by the Bonferroni mean separation test ($P<0.05$).

2). The grower diet fed to all pigs in part 2, contained 83.0% barley, 14.5% soybean meal (47% crude protein), 0.75% dicalcium phosphate, 1.0% limestone with 0.25% trace mineralized salt and 0.50% medicated vitamin and mineral premix providing the identical nutrient supplements to diets in part 1. The experimental starter pig diets were fed for 28 days and the control grower diet was fed for 14 days. At the end of part 1 pigs on 25 and 50% MB had not reached the appropriate weights for a grower diet and therefore required an additional 10 or 14 days on the control starter diet, respectively. Chromic oxide (0.5% of the experimental diets) was used as a marker to determine apparent digestibility coefficients of crude protein and amino acids for part 1 of the study.

The pigs had access to the mash diets and water at all times. Feed consumption per pen and individual weight gains were recorded on days 3 and 7 and weekly thereafter during part 1 of the study. Subsequently (part 2) performance was recorded twice a week. Fecal samples were collected from each pig on day 8 of the experiment (part 1) and subsequently freeze-dried to preserve the nutrients to be measured.

Chemical Analysis

Clean barley, moldy barley and the three experimental diets from part 1 of the experiment were analysed for dry matter, ash, crude protein, crude fat and acid detergent fibre (ADF) according to standard methods of the Association of Official Analytical Chemists (1984). Dietary and fecal proteins were subjected to acid hydrolysis prior to being determined on a LKB 4151 ALPHA PLUS amino acid analyzer. A hydrolysis on 100 mg of air-dried diets or freeze-dried feces was carried out in 4 mL of 6N HCl in test tubes with ground glass joints and glass stopcocks. The air was removed with the aid of a vacuum pump and

the sealed tubes were heated in an autoclaved for 16 hrs at 121°C. Following hydrolysis, the acid was evaporated in a Buchler Instrument Evapo-Mix. The residue was dissolved in 10 mL of a sodium (0.2N) citrate buffer (pH 2.2), filtered through a Whatman number 40 filter paper and stored frozen until analyzed. Before analysis the sample was filtered through a 0.22 μm nylon membrane filter (MSI, Fisher Scientific) and 50 μL were applied to the column. Methionine and cystine were oxidized prior to hydrolysis according to the method of Hirs (1967). Chromic oxide levels in feed and feces were determined by the method of Williams et al. (1962) on a an Instrumentation Laboratory atomic absorption spectrophotometer model 551. The apparent digestibilities of crude protein and amino acids were calculated according to the formula of Maynard et al. (1979). Multitoxin analysis which included an analysis for aflatoxins, ochratoxin A, penicillic acid, citrinin, sterigmatocystin, patulin and zearalenone was done by the methods of Wilson et al. (1976) and Joseffsson and Moller (1977).

Statistical Analysis

A randomized complete block design was used with weight as the blocking variable. About 50% of the animals selected for the experiment were Managra pigs. York and Managra*York breed crosses were represented by about 33 and 17%, respectively. The two breeds and the breed cross were expected to perform similarly. Split-plot analysis for repeated measurements (Snedecor and Cochran 1980) was applied to the individual weight gains, feed intakes per pen and the feed to gain ratios in part 1 of the experiment. A trend over time was explored if a significant treatment x day (of the experiment) or a sex x day interaction occurred. Factorial analysis (Snedecor and Cochran 1980) was done on apparent

crude protein and amino acid digestibility (part 1), overall weight gain, overall feed intake per pen and overall feed to gain ratios (part 2). The initial weight at the start of the experiment as a covariate was also tested, but was found not to significantly contribute to the assumed models. When a significant F-test ($P<0.05$) of the main effects occurred, differences among means were tested using the Bonferroni mean separation test ($P<0.05$) (Neter and Wasserman 1974). The data were analyzed by using the Statistical Analysis System (SAS Institute Inc. 1985).

RESULTS AND DISCUSSION

Although the MB had a higher crude protein content than the clean barley, there was no proportional increase in the amino acid (AA) composition of the MB [Table 2 (for a complete list of AA see Table A1)]. Hence, a higher crude protein content of the diets containing MB resulted in a AA composition similar to the one of the control diet. A higher fibre content of MB increased the fibre content of the diets accordingly (Table 2).

Performance and digestibility were affected by diets containing MB (Table 2). Feed consumption and average daily gains were reduced ($P<0.05$) by 38 and 52%, respectively with 50% MB in the diet. Average daily gain was also depressed ($P<0.05$) by the administration of the diet containing 25% MB. A significant day (of experiment)*treatment ($P<0.05$) and day*sex interaction ($P<0.05$) for average daily gain (ADG) were explored by performing regression analyses of day on ADG for three treatments and two sexes. The shape of the growth curves were found to be different for each treatment. The growth curves for treatment 1 (T_1), T_2 and T_3 were linear, quadratic and cubic, respectively (Fig.

A1). The growth curves for gilts and barrows were both linear, but the rate of gain for gilts was higher until day seven of the experiment (Figure A2). Feed efficiency was not significantly impaired ($P>0.05$) when moldy barley was included in the diet. However, there was a significant day*treatment ($P<0.05$) interaction. A trend over time was explored by evaluating means of feed to gain ratios for the three treatments on day three, seven, fourteen, twenty-one and twenty-eight of the experiment (Figure A3). An influence of MB on feed efficiency was particularly noticed on day three of the experiment and appeared to occur throughout part 1 of the study.

The present findings are in contrast to research from Jones et al. (1955) who did not observe any difference in performance when moldy oats or wheat was incorporated in starter or grower-finisher diets. A higher crude fibre value and lower bushel weight of the moldy oats were signs of lower quality and made it less digestible. Lower bushel weights are generally associated with lower digestible energy values and hence may influence performance negatively. Moldy oats was incorporated at 25% and 36% of the diet from 18-50 kg and 50-90 kg liveweight, respectively. The researchers concluded that performance was not affected due to low levels of moldy oats in the starter and grower-finisher pig diets. MB used in the present experiment also had a higher fibre value, but when it was included at 25% of the diet and was fed to starter pigs in the 16-32 kg liveweight range, performance was depressed. It is not clear why 25% of moldy barley in the diet had an influence on the acceptability of the feed and contrasts with the data of Jones et al. (1955). One reason may be a different grade of moldiness in the two trials. Heavily molded feed will have more of a "moldy smell" caused by

the production of some primary fungal metabolites (Abramson et al. 1980, 1983) and may reduce the acceptability of the feed.

No explanation can be given for the low crude protein and amino acid digestibility from part 1 of the experiment (Table 2) and the data should be used for internal comparison only. Sauer et al. (1977) reported 85.9, 77.5, 79.9, 88.7 and 81.4% for the crude protein, lysine, methionine, cystine and threonine digestibility of barley, respectively. The crude protein digestibility was not affected by the treatments, but the digestibilities of the four amino acids lysine, methionine, cystine and threonine (Table 2) decreased significantly ($P<0.05$) when 50% MB instead of 25% MB was included in the diet (for a complete list of digestibilities see Table A2).

When a control grower diet was fed for two weeks in part 2, normal performance was seen. No significant difference ($P>0.05$) between treatments was found in feed consumption per pen and average daily gain. The overall means ± standard errors for feed consumption per pen and average daily gain were 12.96 ± 0.16 and 0.83 ± 0.02, respectively. Heavier pigs consumed more ($P<0.05$) than lighter pigs. Least square means ±SE for the feed consumption per pen were 13.73 ± 0.23 kg and 12.18 ± 0.23 kg, respectively. Barrows gained significantly more ($P<0.05$) than gilts. A similar trend had been observed in the first part of the experiment. The least square means ± SE of average daily gains for barrows and gilts were 0.90 ± 0.03 kg and 0.77 ± 0.03 kg, respectively. A significant difference between treatments ($P<0.05$) was observed for feed to gain ratios, but due to a small sample size a Bonferroni mean separation test was not able to show any differences. Least square means ± SE for T_1, T_2 and T_3 were 2.65 ± 0.03, 2.40 ± 0.03

and 2.65 ± 0.03, respectively. The feed conversions in this part of the study were better than indicated by NRC (1979) for the 35-60 kg liveweight range.

The present results agree with data from Madsen et al. (1982a) who fed moldy feed for six weeks in the 20-35 kg liveweight range and normal feed in the 35-70 kg liveweight range. Normal performance occurred in the latter part of that study.

In summary, it can be said that moldy toxin-free feed can be administered to weanling pigs for short periods of time, but will result in more days to market and probably poorer feed efficiency. In order to reduce or avoid these problems the following recommendations can be given based on the results obtained in this experiment. Less than 25% MB similar to the kind used in this study could be included in a starter pig diet for a short period of time provided that initially, a low dietary concentration of MB is fed. If no adverse effects on performance are detected, more MB could be included in the diet.

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MANUSCRIPT II: EFFECT OF OCHRATOXIN A CONTAMINATED BARLEY-BASED DIETS ON YOUNG GROWING PIGS

ABSTRACT

The effect of ochratoxin A (OA) contaminated diets was tested in two experiments with young growing pigs. In experiment 3 mycelia containing OA were incorporated into starter mash diets to produce low (LT) (0.5 mg kg^{-1} OA) or high (HT) (2.5 mg kg^{-1} OA) concentrations. The specific treatments (1-4) for two consecutive 21-day periods were: 1-no toxin (NT), NT; 2-NT, HT; 3-LT, NT; 4-LT, HT. In period 3 (21 days) a toxin-free barley-based grower mash diet was fed to all pigs. Feed consumption and feed to gain ratios were not influenced ($P>0.05$) by prior treatments. Average daily gain (ADG) was decreased significantly ($P<0.01$) with the administration of the high OA concentration in period 2. Serum OA levels varied directly with the OA levels in the feed. Two serum parameters, serum total protein and creatinine, were increased significantly ($P<0.05$) even after the administration of a low OA dose. No adaptation from feeding a low OA level and then a high OA level ($P>0.05$) could be found in ADG or the two serum parameters. After the administration of a control grower diet ADG, serum total protein and creatinine approached control values. In summary, pigs fed a low OA level followed by a high OA level (treatment 4) performed no differently than those fed only the high OA level (treatment 2). All pigs were able to recover from the OA levels fed during the early part of the growth period.

Key Words: Ochratoxin A, barley, young growing pigs.

INTRODUCTION

Ochratoxin A (OA) is a dihydro-isocoumarin derivative linked through its 7-carboxy group to L-β-phenylalanine. It is a toxic secondary metabolite of storage fungi such as the Aspergillus or Penicillium species (Chu 1974). Since its first detection on naturally contaminated corn in the United States (Shotwell et al. 1969) it has been found on grains and feeds in Europe and western Canada (Krogh 1978).

Madsen et al. (1982a) demonstrated that performance of market pigs was depressed when OA concentrations in the feed exceeded 0.2 mg kg^{-1}. OA has been detected in blood from slaughter pigs in Poland (Golinski et al. 1984, 1985), Sweden (Hult et al. 1984), West-Germany, (Bauer et al. 1984) and western Canada (Marquardt et al. 1987) and a high correlation has been found between feed, blood and tissue concentrations (Mortensen et al. 1983).

The kidney is the organ most affected at exposure levels, below 5-10 mg kg^{-1} OA in the feed (Krogh 1980) and the degenerative changes of the proximal tubules as well as the glomerulus are described as mycotoxic porcine nephropathy (Elling 1977). Extrarenal effects such as a fatty infiltration of the liver, enteritis, necrosis of the lymphoid tissue and leucocytosis have been observed at higher concentrations (Szczech et al. 1973) and are rarely attributed to naturally contaminated feed.

This study was conducted in order to provide additional data on the effect of OA in barley-based diets on performance, urine and biochemical parameters of young growing pigs. Parameters indicating kidney impairment such as serum total protein, blood urea nitrogen and serum

creatinine were of particular interest.

The fungal contamination of naturally OA contaminated feed was simulated by adding mycelia containing OA to a standard pig diet. This mycotoxin source was evaluated in an initial experiment (experiment 2) and was subsequently used in experiment 3.

Compared to other published research data, starter pigs with a lower initial weight were used in experiment 3. The possible adaptation to OA was studied by initially feeding a low dietary concentration of OA followed by a high dietary concentration of OA. The low or high toxin diets were also tested separately. Subsequent to the toxin-feeding (for 3 or 6 weeks) all pigs were fed a barley-based control grower diet. Information obtained from this study could assist in planning feeding alternatives for commercial pig production where OA contamination in the feed is a problem.

MATERIALS AND METHODS

Source of toxin

Freeze-dried media (FDM) provided OA only, but mycelia supplied the fungal mats including OA. Both OA sources were produced at the same time by surface liquid fermentation for 10 or 12 days at 28°C. The nutrient solution for the fungi contained 4% sucrose, 2% yeast extract (BBL Microbiological Systems, Cockeysville, MD, USA), 1% of L-phenylalanine, 0.25% of L-glutamic acid and L-proline and minerals according to Davis et al. (1972) and Yamazaki et al. (1970). The media were inoculated with well sporulated Aspergillus ochraceus (NRRL 3174) grown on potato dextrose agar with 2% sodium chloride. The mycelial mats were mechanically separated, autoclaved, dried at 50°C and ground with the use of a Cyclotec grinder (1mm screen). The liquid media were

pooled, initially concentrated by a steam evaporator (Cyclone) and finally concentrated to dryness by freeze-drying. Both toxin sources were subjected to a multitoxin analysis which included an analysis for aflatoxins, ochratoxin A, penicillic acid, citrinin, sterigmatocystin, patulin and zearalenone according to the methods of Wilson et al. (1976) and Joseffsson and Moller (1977).

Animals and diets

Experiment 2

Eighteen (18) pigs (Managra, York and Managra x York crosses) with an average starting weight of 35.3±2.6 kg (starting weight ±SD) were randomly allotted to three pens with three barrows and three gilts per pen. Each pen measured 2.60 m x 2.90 m and straw was used as bedding material on the concrete floors.

A basal barley-soybean meal grower pig diet [treatment 1 (T_1)] was formulated to meet or exceed NRC (1979) requirements. Eighty-three percent barley and 14.5% soybean meal (47% crude protein) were mixed with 2.5% of a commercial medicated premix (for a detailed composition of the commercial medicated premix see Table A3). FDM (0.3%) and mycelia (1.4%) were mixed with 99.7% and 98.6% of the basal grower diet, respectively to yield a similar OA concentration. A concentration of 2.7 mg kg^{-1} OA had been calculated for both OA contaminated diets. The three treatments were randomly assigned to the three pens. The experimental mash diets were fed for twenty days. Feed and water were available at all times during this period. Feed consumption per pen and individual weight gains were recorded twice a week.

On day twenty-one each pig was bled from the external jugular vein after a 24-hour fast. Serum was collected immediately from the clotted

blood samples and was divided into two portions. One portion was stored in the refrigerator overnight for immediate analysis of the biochemical parameters. The second portion was frozen at -20°C until analyzed for OA.

Urine samples from each barrow housed in a metabolism cage for a 24-hour period were collected after the blood sampling had taken place. The urine samples were frozen until analysis.

Experiment 3

Thirty-two (32) barrows (Managra and Managra x York crosses) were divided into two weight groups [13.6±1.1 and 16.0±1.8 kg (starting weight ±SD)] and were allotted to eight pens with four barrows per pen. Each pen measured 2.60 m x 1.45 m and straw bedding was used on the concrete floors. Basal barley-soybean starter and grower pig diets were formulated to meet or exceed NRC (1979) requirements. Table 3 shows the composition of the starter pig diet. The composition of the grower pig diet was the same as in experiment 2. Two concentrations of mycelia containing OA (0.2 and 1.0%) were mixed with 99.8 and 99.0% of the basal starter pig diet, respectively. Four treatments (Fig. 3) were randomly assigned to each of the two weight groups. The pigs had access to the mash diets and water at all times. Feed consumption per pen and individual weight gains were measured weekly.

Before the start of the experiment and at the end of each period all the animals were fasted for 24 hours so that blood could be collected from the anterior vena cava. At the beginning two animals per pen were bled, but during the trial blood samples were collected from each animal and two pooled serum samples per pen were prepared. Serum collection and storage procedures were identical to the ones described

Table 3. Composition of the basal starter pig diet - Experiment 3

Ingredients (%)	
Barley	82.0
Soybean meal (47.0%)	15.0
Dicalcium phosphate	1.0
Limestone	1.0
Trace mineralized salt†	0.25
Medicated vitamin and mineral mix‡	0.75

†Trace mineralized salt provided the following nutrients per kg of diet: 2.4 g sodium chloride, 0.25 mg iodine, 0.1 mg cobalt, 4 mg iron, 0.83 mg copper, 3 mg manganese, 10 mg zinc.

‡Medicated vitamin and mineral mix provided the following nutrients per kg of diet: 2200 IU vitamin A, 330 IU vitamin D, 12 μg vitamin B_{12}, 123 mg zinc oxide (88.6 mg zinc min.), 2.5 g mecadox.

FEEDING PERIOD	TREATMENT 1 T_1	TREATMENT 2 T_2	TREATMENT 3 T_3	TREATMENT 4 T_4
FIRST - 21 DAYS	BASAL STARTER DIET	BASAL STARTER DIET	BASAL STARTER DIET + 0.2% MYCELIA + 0.5 mg kg^{-1} OCHRATOXIN A	BASAL STARTER DIET + 0.2% MYCELIA + 0.5 mg kg^{-1} OCHRATOXIN A
SECOND - 21 DAYS	BASAL STARTER DIET	BASAL STARTER DIET + 1.0% MYCELIA + 2.5 mg kg^{-1} OCHRATOXIN A	BASAL STARTER DIET	BASAL STARTER DIET + 1.0% MYCELIA + 2.5 mg kg^{-1} OCHRATOXIN A
THIRD - 21 DAYS	BASAL GROWER DIET	BASAL GROWER DIET	BASAL GROWER DIET	BASAL GROWER DIET

Fig. 3. Feeding scheme - Experiment 3

in experiment 2.

Chemical analysis

For the OA quantification the following extraction procedures were used. Feed or mycelia were ground in the Cyclotec grinder (Tecator ab, Sweden) (1 mm screen) and a 5 g sample was extracted with constant shaking in a 100 mL flask with 2.5 mL of 0.1 M H_3PO_4 and 30 mL of chloroform for 30 minutes. The extract was filtered through Whatman filter (#4) containing 3 g of anhydrous Na_2SO_3. To transfer the residual material from the flask and rinse the filter, 20 mL of chloroform were used. The chloroform was evaporated in a rotary evaporator, the residue was dissolved with an appropriate volume (usually 2-5 mL) of chloroform and the sample was applied to a reverse phase thin layer chromatography (RPTLC) plate, type KC 18F (Whatman, Inc., Clifton, NY 07014). In case of the freeze-dried medium, 1 g of OA containing material was dissolved in 15-18 mL of redistilled water and the pH was adjusted to 2 with 8N HCl. Water was added to a final volume of 20 mL. An aliquot (2 mL) was used for extraction with two 25 mL volumes of chloroform in a separatory funnel. The RPTLC plates were first developed with hexane, then dried and redeveloped with methanol:water (70:30) (v/v), yielding a well-separated OA zone. Visualized under UV light, OA spots were quantitatively recovered from plates, eluted from the RP material with 2 mL of HPLC-grade methanol (Fisher Scientific Ltd., Canada) and evaporated under a stream of nitrogen. Extraction of serum samples for OA was performed by the method of Hult et al. (1979). Quantitative analysis of OA was done by high-performance liquid chromatography (HPLC). Samples were reconstituted with 200-1000 μL of methanol and filtered through 3 mm

membrane nylon filters (Micron Separation Inc., Honeoye Falls, NY 14472) with 0.45 μm pore size. Aliquots of 50 μL were injected into a 250 x 4.6 mm column containing 5 μm diameter C-18 bonded-phase adsorbant (Altex Ultrasphere ODS, Beckman Canada Ltd., Mississauga, Ontario). The mobile phase was methanol:water 70:30 (v/v) and had a flow rate of 1.5 mL/min and a temperature of 50°C. OA was monitored as a well-resolved peak at 4.4 min using a fluorescence detector (Schoeffel FS970, Krotos, Inc., Westwood, New Jersey) with excitation at 333 nm and detection at 418 nm. The detection limit for TLC-HPLC was 0.1 μg/g and the recovery was determined to be 94%.

Biochemical analyses of serum were performed by Dacos (Coulter Electronics Inc., Haileah, FL, USA) using procedures outlined in the company's manual. Analyzed serum enzymes and serum constituents were creatinine kinase (CK) (EC 2.7.3.2), aspartate aminotransferase (AST) (EC 2.6.1.1), alanine aminotransferase (ALT) (EC 2.6.1.2), amylase (EC 3.2.1.1), lipase (EC 3.1.1.3), alkaline phosphatase (ALP) (EC 3.1.3.1), γ-glutamyl transferase (GGT) (EC 2.3.2.2), sodium, potassium, sodium/potassium ratio, chloride, blood urea nitrogen (BUN), creatinine, BUN/creatinine ratio, glucose, total bilirubin (conjugated plus unconjugated), direct bilirubin (conjugated), indirect bilirubin (unconjugated), cholesterol, uric acid, total protein, albumin, globulin, albumin/globulin ratio, phosphorus, calcium and magnesium.

A dip and read test was done on urine for color, sediment, leucocytes, nitrites, pH, protein, glucose, ketones, urobilinogen, bilirubin, blood and hemoglobin with Chemstrip (Boehringer Mannheim, West-Germany).

Statistical analysis

A completely randomized design (experiment 2) and a randomized complete block design (experiment 3) with weight as the blocking variable were used. Managra pigs and Managra*York crossbred pigs were represented by 72 and 22% in experiment 2 and by 31 and 69% in experiment 3, respectively. Six percent of York pigs had been additionally selected for experiment 2. The two breeds and the breed cross were expected to perform similarly. Split-plot analysis for repeated measurements (Snedecor and Cochran 1980) was applied to the individual weight gains (experiment 2 and 3), feed intakes per pen and feed to gain ratios (experiment 3). Factorial analysis was done on feed intakes per pen and feed to gain ratios (experiment 3) as well as on the biochemical blood parameters (experiment 2 and 3). Initial control values in experiment 3 were not included in the analysis, as the variation was high. The initial weight at the start of the experiment as a covariate did not contribute to the models assumed ($P>0.05$). When a significant F-test ($P<0.05$) for main effects occurred, differences among means were tested using the Bonferroni mean separation test ($P<0.05$) (Neter and Wasserman 1974). Significant treatment x period interactions (experiment 3) were explored by the means of contrasts (Steel and Torrie 1980). In the case of significant treatment x period x weight group interactions no test was applied, but rather trends were investigated. The data were analyzed using the Statistical Analysis System (SAS Institute Inc. 1985).

RESULTS AND DISCUSSION

A multi-toxin screening test indicated that OA was the only contaminant to be added to the control diets.

OA levels in the feed

Quantitative analysis of the dietary OA concentration (experiment 2) showed that formulated diets T_2 and T_3 had a concentration of 3.3 and 2.9 mg kg^{-1} OA, respectively and were similar to the calculated value of 2.7 mg kg^{-1} OA.

Performance

A period of three weeks (experiment 2) reduced performance in pigs fed OA containing diets in the 30-50 kg liveweight range (Table 4). Although the average daily gain (ADG) and feed consumption increased significantly ($P<0.05$) over time, the daily feed intake per pen was significantly lower ($P<0.05$) with the addition of OA to the diet. ADG was significantly reduced when the diet was contaminated with mycelia containing OA. The feed conversions were not significantly affected ($P>0.05$) by OA contamination of the feed.

No significant difference was found in the potency of FDM and mycelia. FDM is comparable to crystalline OA and by adding mycelia containing OA to the diet a moldy OA contaminated diet was simulated. In contrast to the present study, Madsen et al. (1982b) compared crystalline OA to OA present in naturally contaminated moldy barley. They found that performance was more depressed by the latter and suggested that fungal odors were partly responsible for the lower acceptability of the feed. Fungal odors are caused by various primary fungal metabolites (Abramson et al. 1980, 1983).

Feed intake and feed conversion increased significantly ($P<0.05$) over time in experiment 3. However, an exposure to a low (0.5 mg kg^{-1}) and/or a high (2.5 mg kg^{-1}) concentration of OA neither decreased feed intake per pen ($P>0.05$) nor reduced ($P>0.05$) feed efficiency

Table 4. Least square means and standard errors of performance for four dietary treatments - Experiment 2 (20 days)

	Dietary treatments (T)			
	T_1 Control	T_2 Freeze-dried media	T_3 Mycelia	SE†
Performance				
Average daily feed consumption/pen (kg)	14.23a	10.29b	9.01b	0.69
Average daily gain (kg)	0.74a	0.46b	0.36b	0.07
Feed/gain ratio	3.33	4.40	5.48‡	0.64

†SE = Standard error.

‡SE = 0.73.

a,b-Least square means with different letters within a row differ significantly by the Bonferroni mean separation test ($P<0.05$).

significantly (Table 5). Treatment*period means for ADG are displayed in Figure 4. A difference in ADG was only noted after feeding a high level of OA. A significant treatment*period interaction for ADG was explored with contrasts of treatment differences in two periods (Figure 5). Feeding a low OA concentration did not result in a significant reduction of ADG ($P>0.05$), but administering a high OA concentration depressed ADG significantly ($P<0.01$). When a control diet replaced the diet contaminated with 2.5 mg kg^{-1} OA, performance values approached control values indicating that no permanent adverse effect on performance had occurred from the earlier toxin feeding. Pigs conditioned with a low concentration of OA did not develop ($P>0.05$) a tolerance for a high concentration of OA (Table 4, Figure 5).

It is not clear why 2.7 mg kg^{-1} OA in the feed depressed feed intake in the 30-50 kg liveweight range (experiment 2), but 2.5 mg kg^{-1} OA in the feed did not affect feed consumption significantly in a similar growth period (experiment 3). Madsen et al. (1982a) reported lower feed intakes when 1.4 and 1.9 mg kg^{-1} OA were fed to pigs in the 20-50 and 50-90 kg liveweight, respectively. The pigs were fed at 85-90% of ad libitum feeding. Feeding the lower OA concentration to younger pigs reduced the daily feed intake by up to 26%, but feeding the higher OA concentration to older pigs caused a reduction of the daily feed allowance by only 4% Krogh et al. (1974) did not observe any change in daily feed intake even after feeding 4.0 mg kg^{-1} OA from 20-90 kg liveweight. However, from 20-50 kg and 50-70 kg liveweight the daily feed allowances were 6-20% and 2-10% lower than the ones described by Madsen et al. (1982a), respectively.

In experiment 3 ADG was not significantly affected by a low OA

Table 5. Least square means and standard errors of feed intake per pen and feed to gain ratios for four dietary treatments - Experiment 3 (9 weeks)

	Dietary treatments (T)				
	T_1 Control	T_2 High toxin	T_3 Low toxin	T_4 Low toxin High toxin	SE†
Performance‡					
Average daily feed consumption/pen (kg)	7.95	7.47	8.17	6.17	0.53
Feed/gain ratio §	2.58	2.78	2.82	2.94	0.04

†SE = Standard error.

‡For average daily gain see Figure 5.

§The treatments were found to be significant ($P<0.05$) in the analysis of variance, but a Bonferroni mean separation test was not able to detect any difference due to a small number of degrees of freedom.

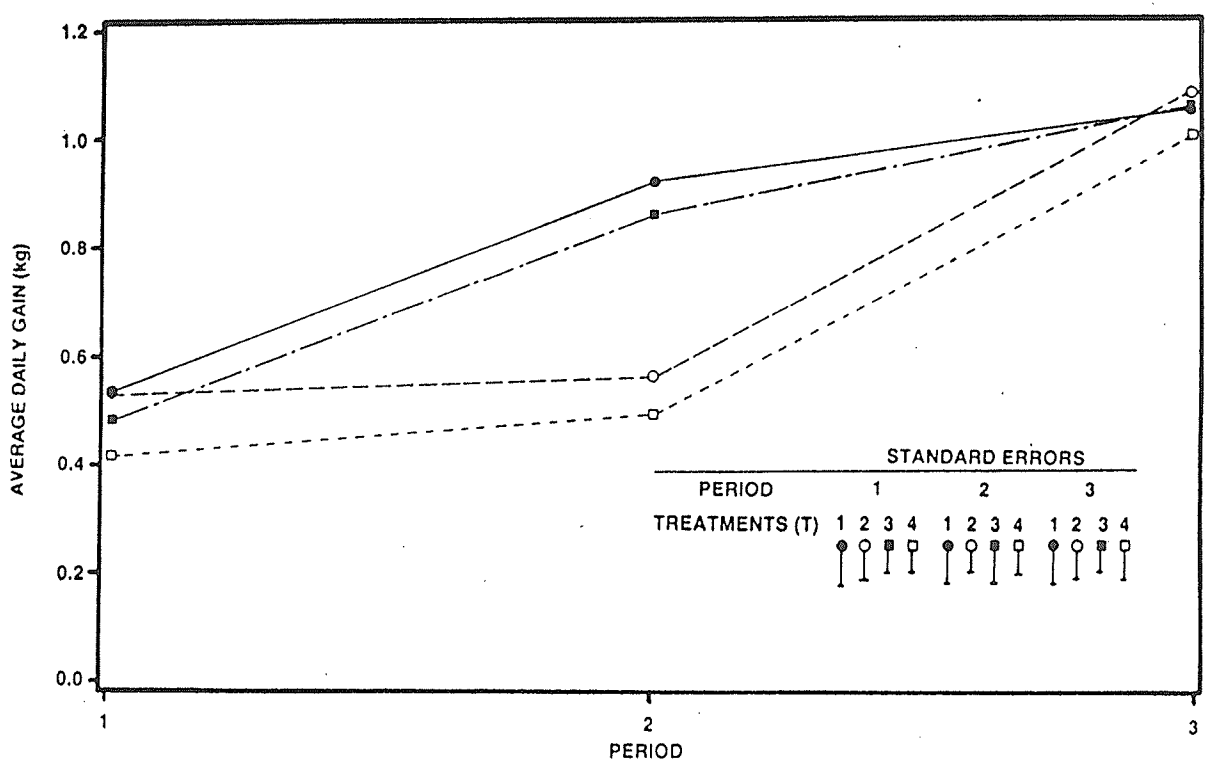


Fig. 4. Means and standard errors for average daily gain in each of four treatments and three periods - Experiment 3.

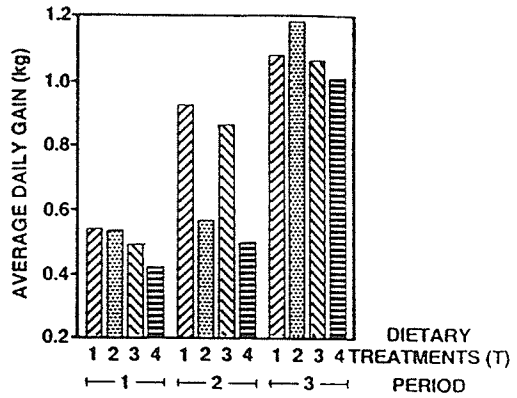
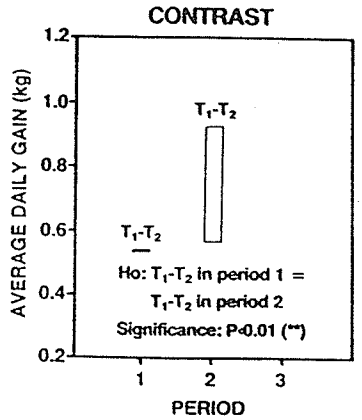


Fig. 5a.



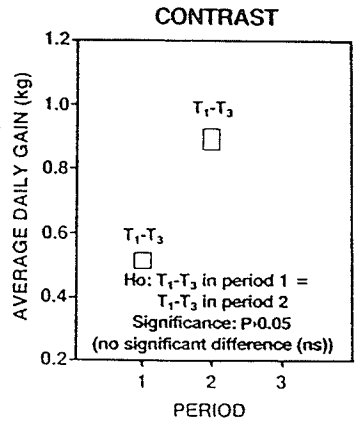
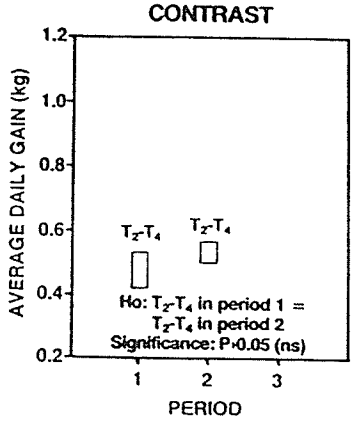


Fig. 5b.

Fig. 5c.



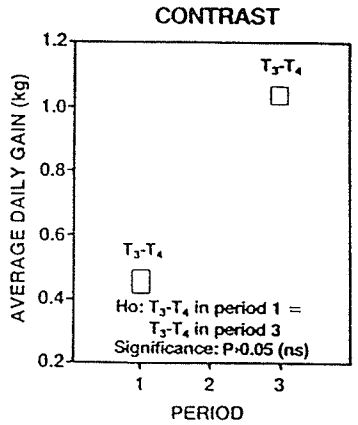


Fig. 5d.

Fig. 5. Treatment differences in two periods for average daily gain (ADG) - Experiment 3; Interpretation: Fig. 5a. A high ochratoxin A (OA) level decreased ADG significantly; Fig 5b. A low OA level did not decrease ADG significantly; Fig. 5c. No adaptation effect from a low to a high OA level was seen; Fig. 5d. ADG approached control values when a high OA diet was succeeded by a toxin-free diet.

concentration in the diet (0.5 mg kg^{-1} OA), but a high dietary OA concentration (2.5 mg kg^{-1}) caused depressed growth when fed for 3 weeks. When concentrations of 0.2, 1.0 and 4.0 mg kg^{-1} OA in the feed were administered in the 20-90 kg liveweight range, reduced weight gains were also noticed at the lowest concentration (Krogh et al. 1974). Krogh et al. (1979) confirmed their earlier results when administering 1.0 mg kg^{-1} OA in the same liveweight range (20-90 kg). Exposures to 1.4 and 1.9 mg kg^{-1} OA during the early (25-50 kg) and later (50-90 kg) growth period, respectively, reduced weight gains significantly (Madsen et al. 1982a).

In another experiment (Madsen et al. 1982a) an OA contaminated diet (1.4 mg kg^{-1} OA) was succeeded by a toxin-free diet in the 35-70 kg liveweight range. Performance was comparable to control values indicating that no permanent adverse effect on performance had occurred from the earlier toxin feeding which agrees with data from experiment 3.

Serum OA levels

Serum OA levels were dependent on dietary OA concentrations. In experiment 3, a low OA contamination of the feed (0.5 mg kg^{-1} OA) resulted in low serum OA residues and higher OA levels were detected after having fed a higher concentration of OA (2.5 mg kg^{-1} OA) in the diets (Figure 6). In experiment 2 only a higher concentration of OA (2.7 mg kg^{-1}) was fed. The serum OA levels were 2253±161 and 3373±253 ng mL^{-1} (mean ± standard error) for pigs on FDM and mycelia, respectively and were similar to the serum OA levels of pigs having received 2.5 mg kg^{-1} OA in the feed. OA contaminated diets had been fed for 3 weeks before serum was analyzed. The OA concentrations in serum were expected to be stable by that time, because a plateau level in the

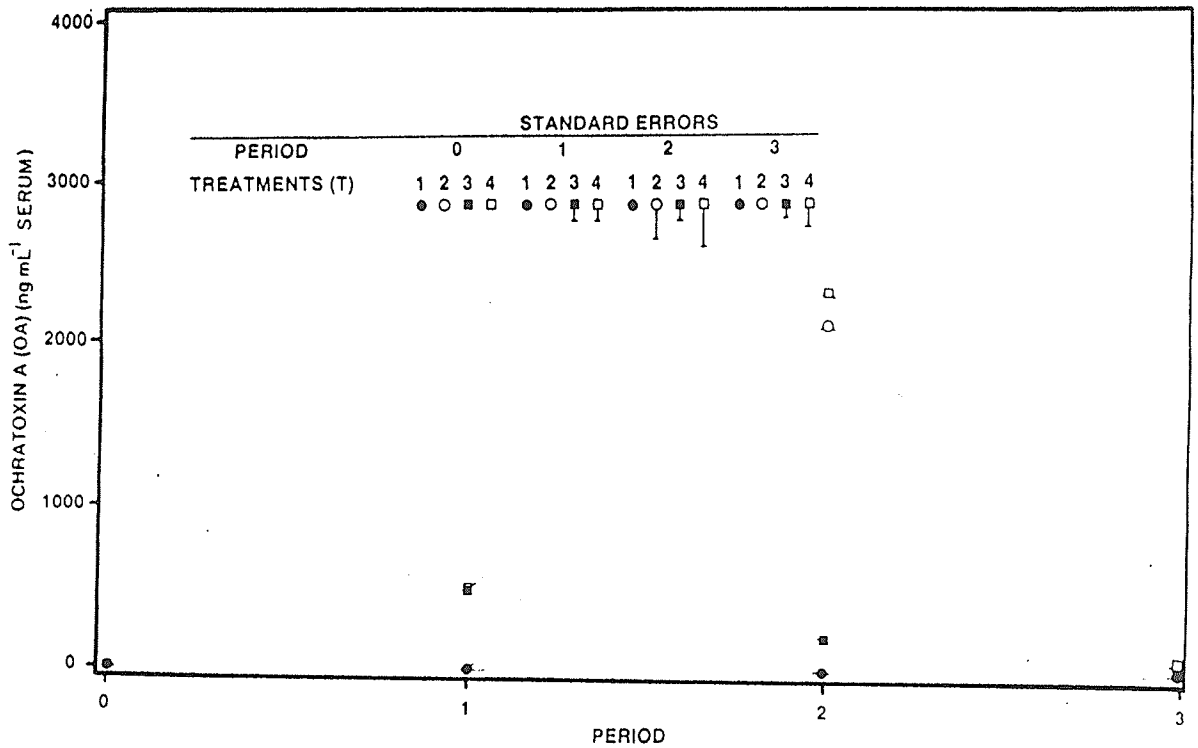


Fig. 6. Serum ochratoxin A concentrations in four treatments over three periods - Experiment 3.

serum is usually approached after two to three weeks on OA contaminated feed (Madsen et al. 1982a, Hult et al. 1979, Mortensen et al. 1983).

A period of three weeks was sufficient to eliminate most of the OA from the serum (Figure 6). This was confirmed by data from Mortensen et al. (1983) who calculated the half-life of OA in serum to be 6.0 days.

Biochemical serum and urine parameters

Serum total protein and serum creatinine were significantly increased ($P<0.05$) after feeding diets contaminated with 2.7 mg kg^{-1} OA in experiment 2 (Table 6). Both parameters are indicators of impaired kidney function and are associated with mycotoxic nephropathy (Szczech et al. 1973). When pigs lose the ability to concentrate urine, they dehydrate easily and this is indicated by increased serum protein levels. Clinical symptoms include polydipsia and polyuria, but water consumption and urine excretion were not measured in the present experiment. Increases in serum creatinine indicate a reduced glomerular filtration rate (Coles 1974), a condition which is observed in porcine mycotoxic nephropathy.

Decreased ($P<0.05$) serum glucose levels and increased ($P<0.05$) globulin levels were also a result of the addition of OA to the diet in the second trial (Table 6). Although the treatments were significantly different for calcium as well, a Bonferroni mean separation test ($P<0.05$) was not able to show any differences due to the small sample size. Decreased glucose levels may result from a normal hepatic glucose output with an increased peripheral uptake or a decrease in hepatic gluconeogenesis with normal peripheral utilization or a combination of both (Coles 1974). However, feeding of mycotoxins including OA have been associated with increased serum glucose concentrations and reduced

Table 6. Least square means and standard errors of serum parameters for four dietary treatments - Experiment 2 (21 days)

	Dietary treatments (T)			
	T_1 Control	T_2 Freeze-dried media	T_3 Mycelia	SE†
Serum parameters				
Glucose (mmol L^{-1})	5.5a	4.7b	4.7b	0.2
Creatinine (mmol L^{-1})	0.11a	0.14b	0.15b	0.01
Total protein (g L^{-1})	71a	79b	79b	2
Globulin (g L^{-1})	30.8a	36.2b	38.4b	1.5
Calcium‡ (mmol L^{-1})	2.78	2.76	2.88	0.03

†SE = Standard error.

‡The treatments were found to be significant in the analysis of variance but a Bonferroni mean separation test ($P<0.05$) was not able to detect any difference due to a small sample size.

a,b-Least square means with different letters within a row differ significantly by the Bonferroni mean separation test ($P<0.05$).

hepatic glycogen levels (Hsieh 1979). Therefore it is not clear why the glucose levels were lower in the present experiment. Coles (1974) related diffuse fibrosis and hepatitis to an increased serum globulin level. A further partitioning into the various globulin fractions would be necessary to identify a specific cause.

Gilts had significantly higher ($P<0.05$) serum creatinine and calcium levels than barrows. Overall means of serum parameters not influenced by the treatments are presented in Table A4. Uric acid and lipase were not detected. Total bilirubin, direct bilirubin and indirect bilirubin were not included in the statistical analysis, as too many values were unreliable due to hemolysis in some samples.

Serum total protein, serum creatinine, serum glucose and serum globulin were equally affected by FDM or mycelia ($P>0.05$). In order to again simulate naturally contaminated feed, mycelia was chosen as contaminant for the third trial.

A significant treatment x period interaction of serum total protein and serum creatinine, indicators of kidney function, was explored with contrasts of treatment differences in two periods (Figure 7, 8). Both a low (0.5 mg kg^{-1} OA) and a high (2.5 mg kg^{-1} OA) dietary OA contamination resulted in a significant increase ($P<0.05$) of serum total protein and creatinine. When a toxin-free diet replaced the OA contaminated diets, serum total protein and serum creatinine levels approached control values ($P>0.05$) indicating that no permanent damage on kidney function had occurred. Pigs that had been conditioned with a low OA concentration did not develop ($P>0.05$) a tolerance towards a high OA concentration.

A significant treatment x period x weight group interaction

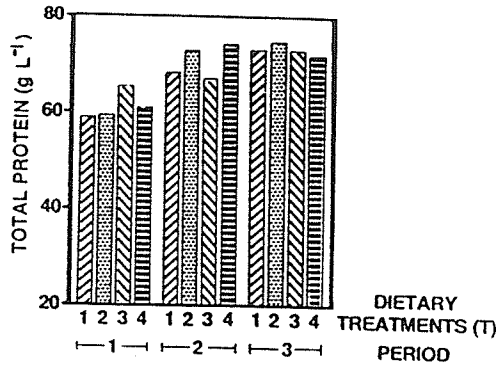
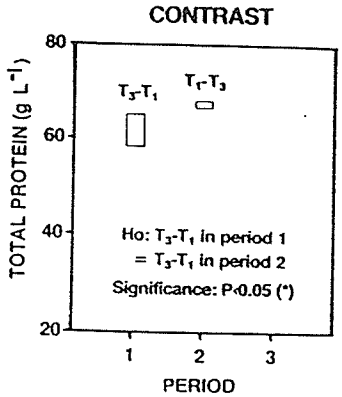


Fig. 7a.



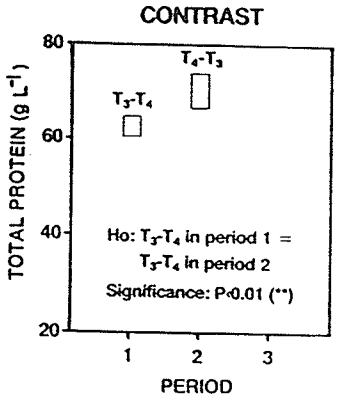
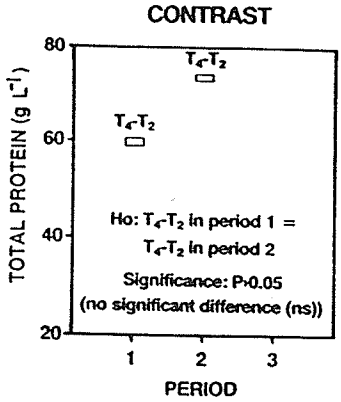


Fig. 7b.

Fig. 7c.



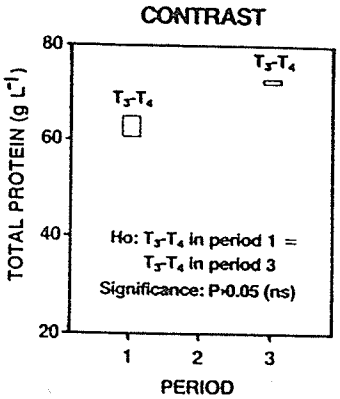


Fig. 7d.

Fig. 7. Treatment differences in two periods for serum total protein-Experiment 3; Interpretation: Fig. 7a. A low level of ochratoxin A (OA) increased the serum total protein levels significantly in period 1; the serum total protein levels decreased to levels found in the control group after a toxin-free period; Fig. 7b. A high OA level increased serum total protein levels significantly; Fig. 7c. No adaptation effect from a low to a high OA level was seen; Fig. 7d. Serum total protein levels approached control values when a high OA diet was succeeded by a toxin-free diet.

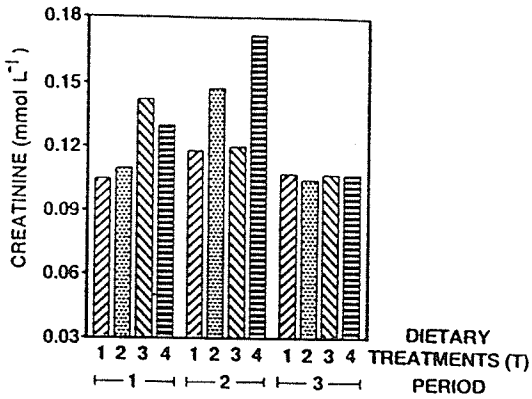
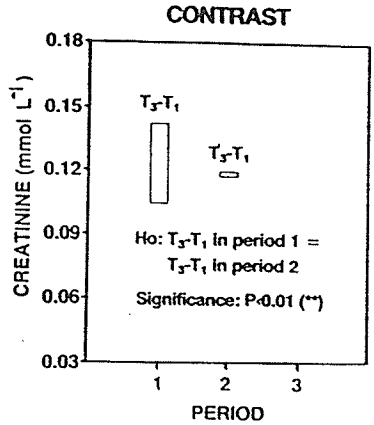


Fig. 8a.



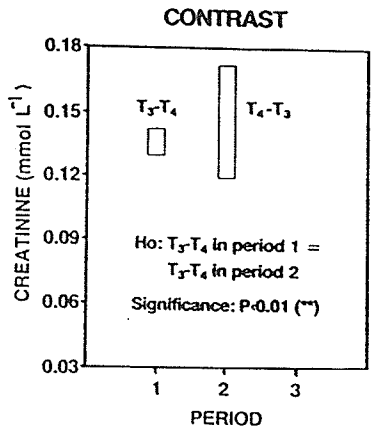
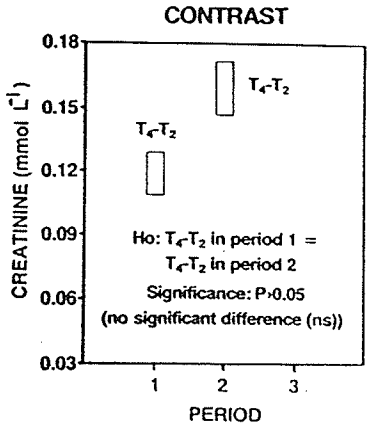


Fig. 8b.

Fig. 8c.



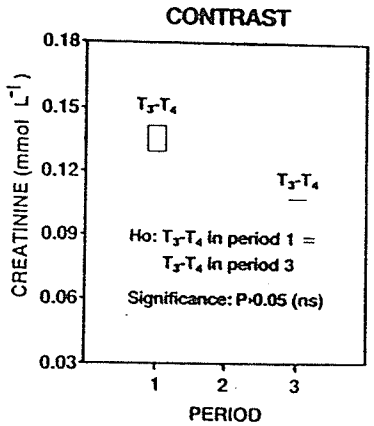


Fig. 8d.

Fig. 8. Treatment differences in two periods for serum creatinine - Experiment 3; Interpretation: Fig. 8a. A low level of ochratoxin A (OA) increased the serum creatinine levels significantly in period 1; the serum creatinine levels approached levels found in the control group after a toxin-free period; Fig. 8b. A high OA level increased serum creatinine levels significantly; Fig. 8c. No adaptation effect from a low to a high OA level was seen; Fig. 8d. Serum creatinine levels approached control values when a high OA diet was succeeded by a toxin-free diet.

occurred in the case of blood urea nitrogen (BUN), BUN/serum creatinine ratio, serum glucose and chloride levels. There seemed to be an indication that lighter pigs were more affected by the addition of toxin to the diet. This trend was especially noticeable in the second period (see Figure A4, A5, A6, A7).

GGT, ALP and calcium were affected by treatments ($P<0.05$) (Table 7). Pigs after having been exposed to high OA levels in the feed had significantly higher calcium levels than pigs on a control diet.

Parameters not affected by the treatments are listed in Table A5. Most of them increased ($P<0.05$) over time. GGT, albumin, albumin/globulin ratio, total protein, creatinine, potassium and sodium/potassium ratio were found to be significantly lower ($P<0.05$) in lighter pigs than in heavier pigs.

The dip and read test for the urine parameters (Table A4) was only performed in experiment 2 and did not reveal any major changes. Since protein and blood were also detected in the urine of some or all the control pigs, contamination of the urine during the collection process was assumed. Detection of protein (Krogh et al. 1974, 1979, Szczech et al. 1973, Zimmermann et al. 1979, Tapia and Seawright 1984) and blood (Zimmermann et al. 1979) are normally a sign of impaired renal function.

From this experiment there is evidence to suggest that there is no benefit in feeding increasing OA concentrations over time. Pigs can be exposed to 2.5 mg kg^{-1} OA during a short period of time, but lower performance and maybe even permanent damage of the kidney tissues (Elling 1983) may be the result. At least one month before slaughter "clean" feed should replace toxic feed so that OA residues can be eliminated. These were the first trials with pigs in Manitoba using

Table 7. Least square means and standard errors of serum parameters for four dietary treatments - Experiment 3 (9 weeks)

	Dietary treatments (T)				
	T_1 Control	T_2 High toxin	T_3 Low toxin	T_4 Low toxin High toxin	SE†
Serum parameters					
γ-Glutamyl transpeptidase [GGT] (U L^{-1})	43a	34b	34b	39ab	2
Alkaline phosphatase [ALP] (U L^{-1})	285ab	241a	312b	273ab	14
Calcium (mmol L^{-1})	2.65a	2.80b	2.75ab	2.80b	0.03

†SE = Standard error.

a,b-Least square means with different letters within a row differ significantly by the Bonferroni mean separation test ($P<0.05$).

mycelia in order to simulate naturally contaminated feed. Compared to other published data, more serum parameters were affected by OA contamination of the feed. Additional research is needed to verify the present results.

ACKNOWLEDGEMENTS

The authors thank Karen Lim, John Gano, The University of Manitoba Swine Barn staff and various graduate students for their technical assistance. The financial support of the Natural Sciences and Engineering Research Council of Canada (Strategic Grant) is acknowledged.

GENERAL DISCUSSION

Ochratoxin A (OA), a mycotoxin, is associated with the formation of storage fungi such as Aspergillus ochraceus Wilhelm (Van der Merwe et al. 1965) or Penicillium verrucosum var. cyclopium (Westling) Samson, Stolk and Hadlock (Abramson and Mills 1985). However, invasion of grain by storage fungi does not necessarily result in mycotoxin production.

Both the administration of moldy toxin-free and OA contaminated feed were studied. The consequences of feeding an OA contaminated diet to pigs have been studied by several groups (Szczech et al. 1973, Krogh 1978, Krogh et al. 1974, 1976, 1979, Madsen et al. 1982a). However, the question of possible adaptation to OA has not been raised so far and we decided to study it.

Performance was affected by moldy and OA contaminated diets. When 50% moldy mycotoxin-free barley (MB) was included in starter pig diets, feed intake and average daily gain (ADG) were depressed. A 25% MB diet had less effect depressing gains only. The digestibility of most amino acids was negatively influenced ($P<0.05$) with the higher MB concentration in the feed. In the third trial feed intake per pen was not significantly affected ($P>0.05$) by the OA contaminated diets, but ADG was significantly depressed ($P<0.05$) by the high OA concentration. Feed efficiencies were not significantly different ($P>0.05$) in both trials after the administration of the experimental diets. In order to compare the results of experiment 3 with the results of experiment 1, only six weeks of data should be considered in the third experiment, because mycelia containing toxin was only fed for up to six weeks. When the statistical analysis of the third trial was done on six weeks of data (Table A5, Figure A8), the results were the same with one

exception. Pigs that had received both low and high OA concentrations had significantly poorer feed conversions ($P<0.05$) than pigs on the other treatment groups.

In the first trial a 16 or 38% reduction in feed intake resulted in a 28 or 53% reduction of ADG, respectively. Since a larger reduction occurred in ADG than in feed intake, not all of the depression in gain can be attributed to the lower feed intake. Various primary fungal metabolites responsible for the "moldy smell" of the MB (Abramson et al. 1980, 1983) or other yet unidentified factors (Madsen et al. 1982b) may have lowered the acceptability and utilization of the moldy feed and once ingested must have further affected the weight gains. In the third trial the difference between depression in ADG and feed intake per pen was not as large as in the first trial although two antinutritional factors (mycelia and OA) had been added to the diets. However, diets contaminated with mycelia containing OA were only administered during the first two periods and a toxin-free ration was fed during the last period. When the last toxin-free period was excluded from the calculations, the differences between depression in feed intake and gains approached those of the first trial.

Naturally contaminated moldy mycotoxin-free barley was used in the first trial and mycelia (containing OA) was added to a control diet in the third trial to simulate naturally contaminated feed. Mycelia (containing OA) grown naturally on barley may be linked to the grain kernel whereas mycelia (containing OA) mixed with high quality feed may be considered one of two separate entities and may therefore have a different effect on the animal. In the second experiment there was no difference in performance ($P>0.05$) between mycelia containing OA and

freeze-dried media (FDM) which is similar to crystalline OA. When Madsen et al. (1982b) compared naturally contaminated feed to control feed contaminated with crystalline OA, they found that naturally contaminated feed depressed performance more. Therefore naturally contaminated feed may have more depressing effects on performance than mycelia containing OA. In order to best compare the three experiments moldy barley naturally contaminated with OA should have been used in the second and third trial.

When moldy toxin-free or OA contaminated feed was replaced by a control diet in the first and third experiment, respectively, performance approached control values indicating that no permanent damage on performance had occurred. This is in agreement with results from Madsen et al. (1982a). No compensatory growth occurred in the present study, because growth did not exceed normal values.

Serum OA levels and biochemical parameters were monitored during the administration of OA in the second and third experiment. Serum OA concentrations reflected the feed OA concentrations. A high correlation has been found between OA administration in the feed and the OA appearance in the serum (Mortensen et al. 1983). Hult et al. (1980) related the OA content in plasma to feed OA concentrations, but referred to a modification of the formula if serum instead of plasma was analyzed. Serum creatinine and total protein, both indicators of kidney impairment occurring in porcine mycotoxic nephropathy (Szczech et al. 1973), were significantly increased ($P<0.05$), even after a low OA dose. Pigs did not develop a tolerance towards a high concentration of OA when they had been conditioned with a low concentration of OA. This was demonstrated in ADG, serum creatinine and total protein. Compared to

other published data, additional serum parameters affected ($P<0.05$) by the administration of OA were γ-glutamyl transpeptidase (GGT), alkaline phosphatase (ALP), calcium, blood urea nitrogen (BUN), BUN/serum creatinine, chloride and glucose. Serum GGT and ALP increase in liver disease (Kramer 1980). BUN and BUN/serum creatinine ratio are used to detect renal dysfunction (Finco 1980). Alterations of serum calcium and chloride concentrations are signs of imbalances in calcium/phosphorus (Simesen 1980) and electrolyte metabolism (Tasker 1980), respectively. Serum glucose levels depend on the supply and removal of glucose by the liver (Kaneki 1980). More research, however, is needed to confirm the results obtained in the present experiment, because in comparison, Szczech et al. (1973) fed very high OA concentrations, but apart from indicators of kidney impairment only one enzyme [glutamic oxaloacetic transaminase (GOT)] showed increased activities. A higher serum GOT level is an indication of a liver dysfunction only in conjunction with the increase of liver specific enzymes (Kramer 1980).

Briefly, it can be said that both moldy mycotoxin-free and toxic feed reduced performance and are therefore undesirable as feedstuffs. Spoilt feed can be diluted, but a commercial pig producer would have to consider the costs of more days to market and poorer feed efficiency. Ideally, market pigs should have access to high quality feed at all times.

SUMMARY AND CONCLUSIONS

The effects of moldy mycotoxin-free (MB) and ochratoxin A (OA) contaminated diets on market pigs were studied in three experiments. Feed intake per pen was lowered ($P<0.05$) when 50% MB and 2.7 mg kg^{-1} OA were incorporated into starter or grower pig diets. Average daily gain was significantly impaired ($P<0.05$), but feed efficiency was not significantly reduced after the administration of MB, 2.5 or 2.7 mg kg^{-1} OA. Most of the amino acid digestibilities were decreased ($P<0.05$) with 50% MB. Succeeding a MB or OA contaminated diet with a control diet resulted in a return to normal performance.

None of the urine parameters showed any major abnormalities after the administration of 2.7 mg kg^{-1} OA in the diet. Higher than normal levels of serum creatinine and serum total protein, signs of kidney damage as observed in porcine mycotoxic nephropathy, were measured after the administration of 0.5, 2.5 and 2.7 mg kg^{-1} OA in the diet. Serum glucose levels were significantly decreased ($P<0.05$) and serum globulin levels were significantly increased ($P<0.05$) by the feeding of 2.7 mg kg^{-1} OA in the diet. With the addition of 0.5 and/or 2.5 mg kg^{-1} OA to the diet, decreased γ-glutamyl transpeptidase activities and increased calcium levels were observed. Calcium values increased significantly ($P<0.05$) when 2.5 mg kg^{-1} OA had been fed. There were no definite trends in the case of alkaline phosphatase.

From the experiments the following conclusions can be drawn:

1. Both MB concentrations (25 or 50% of a starter pig diet) or OA concentrations (2.5, 2.7 mg kg^{-1} OA of a starter or grower pig diet) can be administered for a limited amount of time to market pigs, but will result in more days to market.

2. Lower concentrations of both MB (<25%) or OA (<0.5 mg kg^{-1} OA) may be fed for a short period of time without any major adverse effects on performance.

3. Since pigs in this experiment did not adapt when fed a low OA level and then a high OA level, there is no benefit in supplying OA contaminated feed at increasing levels over a period of time.

4. More serum parameters than have been reported by other researchers were affected by the administration of freeze-dried media containing OA or mycelia containing OA.

Future research could include the following:

1. A lower MB concentration for starter pigs or a higher MB concentration for grower or finisher pigs could be tested in order to determine a concentration which could be safely fed to pigs.

2. Mycelia containing OA should be compared to naturally OA contaminated feed to evaluate possible differences.

3. Serum parameters should be monitored again after the administration of FDM containing OA, mycelia containing OA and naturally OA contaminated barley, but samples should be taken more frequently in order to observe any changes more closely.

4. Urine should be collected with a catheter in order to avoid contamination and subjected to a more intense examination.

5. Determining a method of detoxifying OA contaminated feed and the effect on market pigs should be further explored.

6. For statistical purposes, individual feeding of pigs should be encouraged and the number of pigs used increased.

LITERATURE CITED

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A P P E N D I X

Table A1. Amino acid analysis of clean Bedford barley, moldy Bedford barley and experimental diets

	Clean Bedford Barley	Moldy Bedford Barley	Experimental Diets 0% MB† T_1	25% MB T_2	50% MB T_3
Amino Acids (%)					
Indispensable					
Arginine	0.57	0.61	1.10	1.19	1.14
Lysine	0.43	0.40	0.82	0.84	0.79
Histidine	0.35	0.53	0.48	0.59	0.59
Threonine	0.36	0.45	0.60	0.62	0.61
Methionine	0.28	0.27	0.30	0.34	0.34
Isoleucine	0.44	0.53	0.73	0.75	0.73
Leucine	0.82	0.97	1.28	1.30	1.27
Phenylalanine	0.67	0.76	0.93	0.95	0.91
Valine	0.63	0.72	0.93	0.95	0.92
Dispensable					
Aspartic Acid	0.72	0.83	1.37	1.50	1.48
Serine	0.87	0.51	0.68	0.72	0.69
Glutamic Acid	2.62	2.84	3.67	3.52	3.35
Proline	1.21	1.32	1.60	1.52	1.41
Glycine	0.46	0.58	0.73	0.76	0.74
Cystine	0.31	0.30	0.34	0.38	0.38
Tyrosine	0.33	0.33	0.46	0.46	0.46
Alanine	0.46	0.58	0.82	0.79	0.78

†MB = Moldy Barley

Table A2. Amino acid digestibilities for three treatments during the first part of the moldy barley experiment

Digestibility (%)†	Experimental Diets 0% MB‡	25% MB	50% MB	SE
Indispensable				
Arginine	74.8ab	76.9a	71.3b	1.14
Lysine	46.6ab	52.8a	41.7b	2.32
Histidine§	75.8	79.1	75.2	1.11
Threonine	51.1ab	56.6a	48.2b	2.11
Methionine	54.7ab	56.7a	45.4b	2.58
Isoleucine	54.2ab	58.6a	50.5b	2.07
Leucine	61.4ab	64.0a	56.3b	1.77
Phenylalanine	66.6ab	68.2a	60.8b	1.69
Dispensable				
Aspartic Acid	55.5a	63.2b	56.5a	1.84
Serine	65.2a	67.7a	60.1b	1.55
Glutamic Acid	77.1ab	77.8a	73.1b	1.20
Proline	79.4ab	81.0a	75.6b	1.16
Glycine	54.5ab	58.0a	49.1b	2.10
Cystine	77.3a	76.0a	69.9b	1.21
Tyrosine	55.9ab	59.3a	48.8b	2.21

†Overall digestibility ±SE of valine and alanine are 58.5±1.2% and 42.1±1.6%, respectively.

‡MB = Moldy Barley; SE = Standard error of the mean.

§The treatments were found to be significant in the analysis of variance but a Bonferroni mean separation test ($P<0.05$) was not able to detect any difference due to a small sample size.

a,b Least square means with different letters within a row differ significantly by the Bonferroni mean separation test ($P<0.05$).

Table A3. Composition of the commercial medicated premix (per kg of diet)

Calcium (g)	5.9
Phosphorus (g)	2.3
Sodium chloride (g)	3.5
Sodium (g)	1.5
Copper (mg)	10.0
Iron (mg)	100.0
Zinc (mg)	105.0
Manganese (mg)	27.5
Magnesium (mg)	15.0
Cobalt (mg)	0.5
Iodine (mg)	2.3
Vitamin A (IU)	9,000
Vitamin D (IU)	1,500
Vitamin E (IU)	18
Vitamin K (mg)	2.2
Choline (mg)	262.5
Niacin (mg)	36.3
Pantothenic acid (mg)	11.3
Riboflavin (mg)	4.5
Thiamine (mg)	1.0
Pyridoxine (mg)	1.0
Vitamin B_{12} (μg)	13.8
Selenium (mg)	0.1
Chlortetracycline hydrochloride (g)	22.0

Table A4. Means and standard errors of serum parameters not affected by dietary treatments - Experiment 2

Minerals	
Sodium (mmol L^{-1})	142.2±1.1
Potassium (mmol L^{-1})	5.08±0.22
Chloride (mmol L^{-1})	97±1
Phosphorus (mmol L^{-1})	3.09±0.08
Magnesium (mmol L^{-1})	1.21±0.03
Enzymes	
Amylase (U L^{-1})	1194±95
Creatinkinase [CK] (U L^{-1})	1814±550
Aspartate amino transferase [AST] (U L^{-1})	43±3
Alanine amino transferase [ALT] (U L^{-1})	49±2
γ-Glutamyl transpeptidase [GGT] (U L^{-1})	33±2
Alkaline phosphatase [ALP} (U L^{-1})	215±14
Proteins	
Albumin (g L^{-1})	41.0±0.7
Blood urea nitrogen (mmol L^{-1})	7.4±0.3
Cholesterol (mmol L^{-1})	2.39±0.3

Table A5. Change of serum parameters over time (Experiment 3)

Parameters†	Period 1	2	3	SE
Minerals				
Sodium (mmol L^{-1})	138.3a	141.4b	147.3c	0.55
Potassium (mmol L^{-1})	6.67ab	6.46a	7.36b	0.20
Calcium (mmol L^{-1})	2.68a	2.76ab	2.80b	0.02
Enzymes				
Creatinkinase [CK] (U L^{-1})	1157a	964a	3844b	553
Aspartarte amino transferase [AST] (U L^{-1})	56ab	44a	110b	16
Alanine amino transferase [ALT] (U L^{-1})	37a	44a	59b	3
γ-Glutamyl transpeptidase [GGT] (U L^{-1})‡	42	36	35	2
Alkaline phosphatase [ALP] (U L^{-1})	298a	287ab	249b	12
Proteins				
Albumin (g L^{-1})	28.1a	33.6b	38.7c	0.7
Globulin (g L^{-1})	33.2a	37.2b	34.7ab	0.7
Albumin/globulin ratio	0.85a	0.91a	1.13b	0.04

†Overall mean ±SE of cholesterol and phosphorus 2.62±0.04 and 3.54±0.08 mmol L^{-1}, respectively; SE = standard error.

‡The treatments were found to be significant ($P<0.05$) in the analysis of variance, but a Bonferroni mean separation test was not able to detect any difference due to a small number of degrees of freedom.

a-c-Least square means with different letters within a row differ significantly by the Bonferroni mean separation test ($P<0.05$).

Table A6. Dip and read test for urine parameters from eight barrows with chemistrip (Boehringer Mannheim)

	Dietary treatments							
	T_1			T_2†				T_3
Pig number	723	823	786	971	894	923	1013	901
Color	Light yellow							
Sediment (neg/pos)	Pos‡	Pos	Neg	Pos	Pos	Pos	Pos	Pos
Leucocytes (Leuco μL^{-1})	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg
Nitrite (neg/pos)	Pos	Pos	Pos	Pos	Pos	Pos	Pos	Pos
pH	9	6	6	6.5	7	6.5	8.5	6.5
Protein (gL^{-1})	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace
Glucose (mg L^{-1})	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal
Ketones (neg/pos)	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg
Uroblinogen ($\mu mol\ L^{-1}$)	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal
Bilirubin (neg/pos)	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg
Blood (ERY§ μL^{-1})	Neg	250	5-10	5-10	10-50	10-50	50-250	50
Hemoglobin (ERY μL^{-1})	Neg	10-50	<10	<10	10	10	50	10

†One sample from T_2 had to be discarded because of a high grade of contamination.

‡Pos = positive; neg = negative.

§ERY = Erythrocytes.

Table A7. Least square means and standard errors of performance for four dietary treatments - Experiment 3 (6 weeks)

	Dietary treatments (T)				
	T_1	T_2	T_3	T_4	SE†
Performance‡					
Average daily feed consumption/pen (kg)	6.63	5.60	6.55	4.98	0.50
Feed/gain ratio	2.46a	2.87a	2.70a	3.08b	0.06

†SE = Standard error.

‡For average daily gain see Figure A8.

a,b-Least square means with different letters within a row differ significantly by the Bonferroni mean separation test ($P<0.05$).

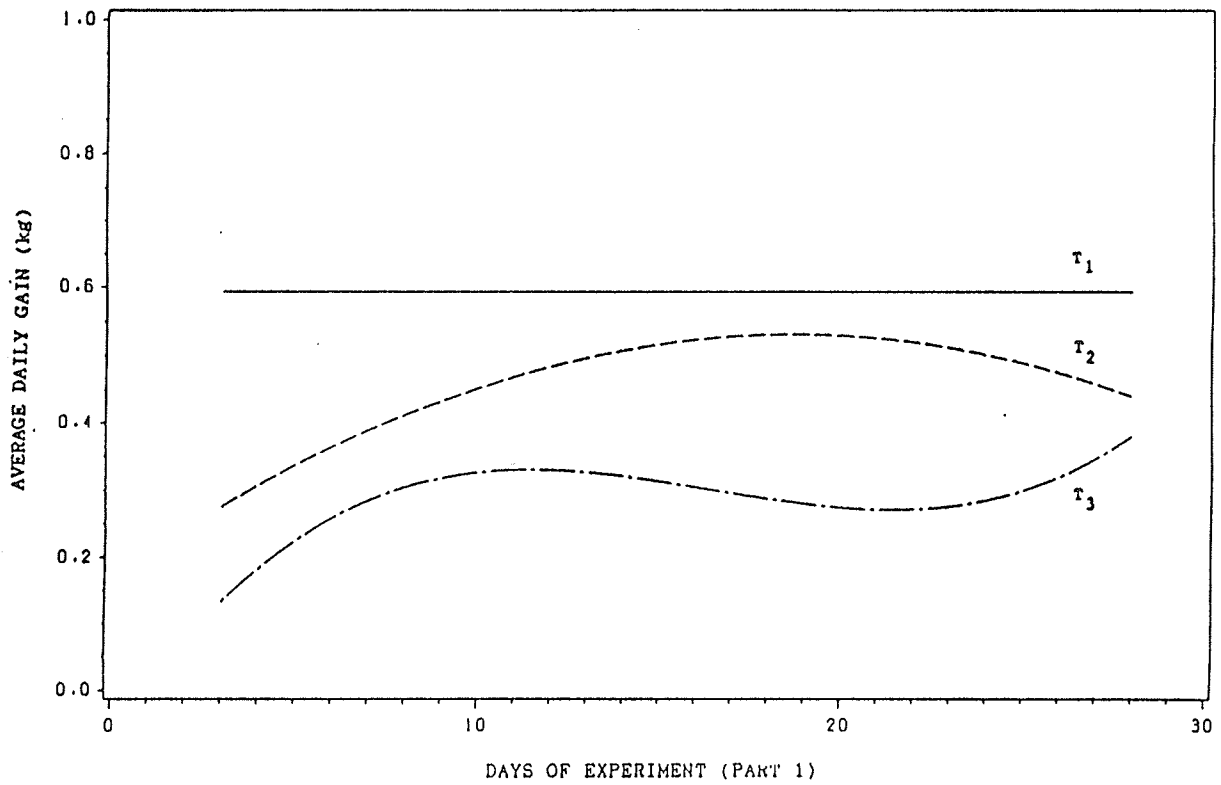


Fig. A1. Influence of moldy barley levels in feed on pattern of average daily gain in a 28-day trial with swine (experiment 1, part 1); —— 0% moldy barley (MB)=treatment 1 (T_1), AVERAGE DAILY GAIN (ADG)=0.59; - - - 25% MB=T_2, ADG=0.17+0.039*DAY-0.0010*DAY^2 (R^2=0.33); -·-·- 50% MB=T_3, ADG=0.08+0.087*DAY-0.0058*DAY^2+0.00012*DAY^3 (R^2=0.33).

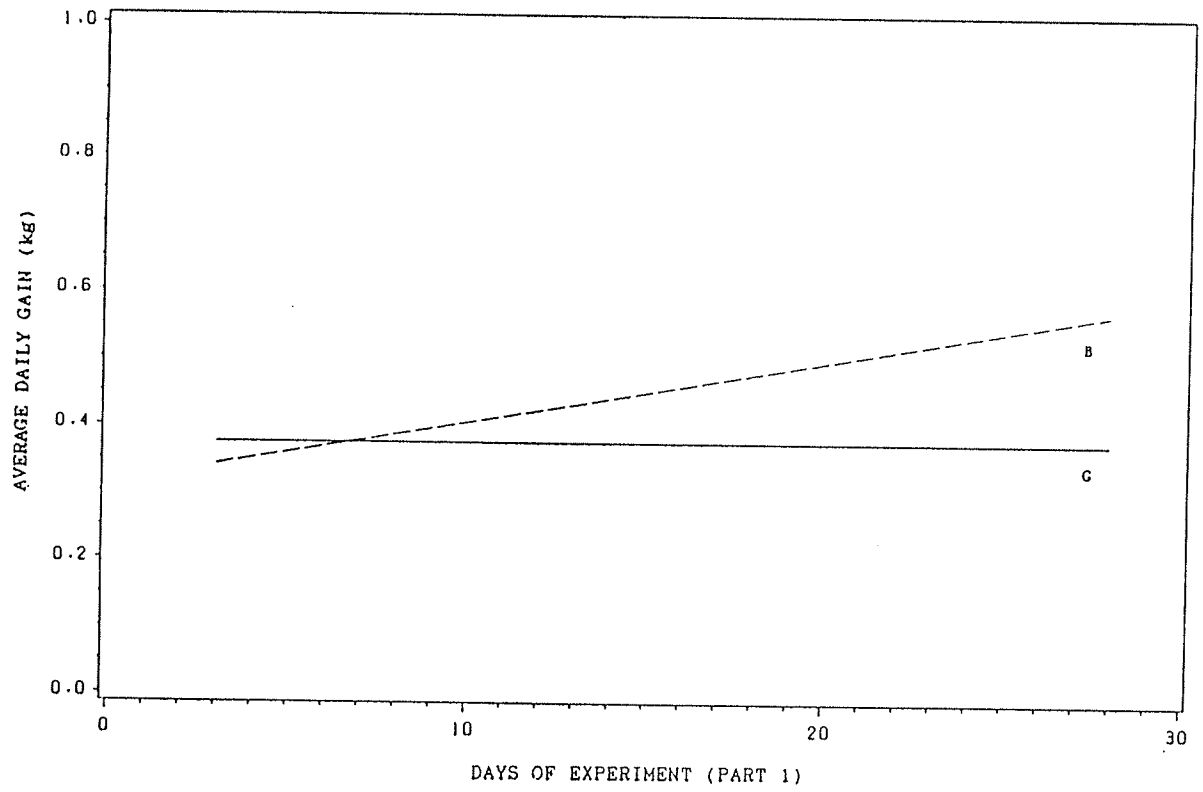


Fig. A2. Influence of sex on pattern of average daily gain in a 28-day trial with swine (experiment 1, part 1); —— gilts (G) AVERAGE DAILY GAIN (ADG)=0.38;---barrows (B) ADG=0.32+0.01*DAY (R^2=0.17).

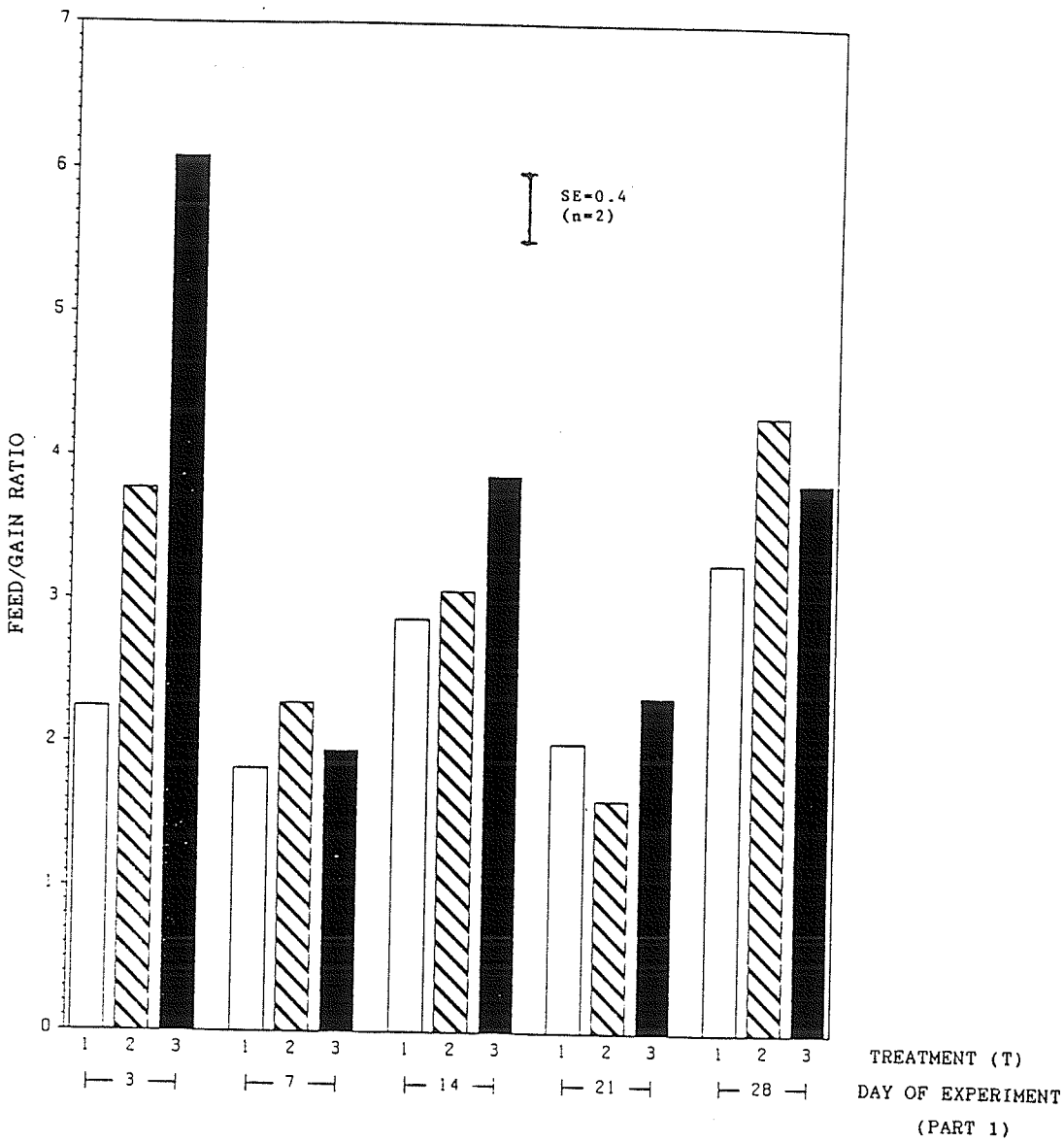


Fig. A3. Influence of three treatments (T) [0% moldy barley (MB)=T_1, 25% MB=T_2, 50% MB=T_3] on means of feed/gain ratios at day three, seven, fourteen, twenty-one and twenty-eight of experiment 1 (part 1); SE=standard error.

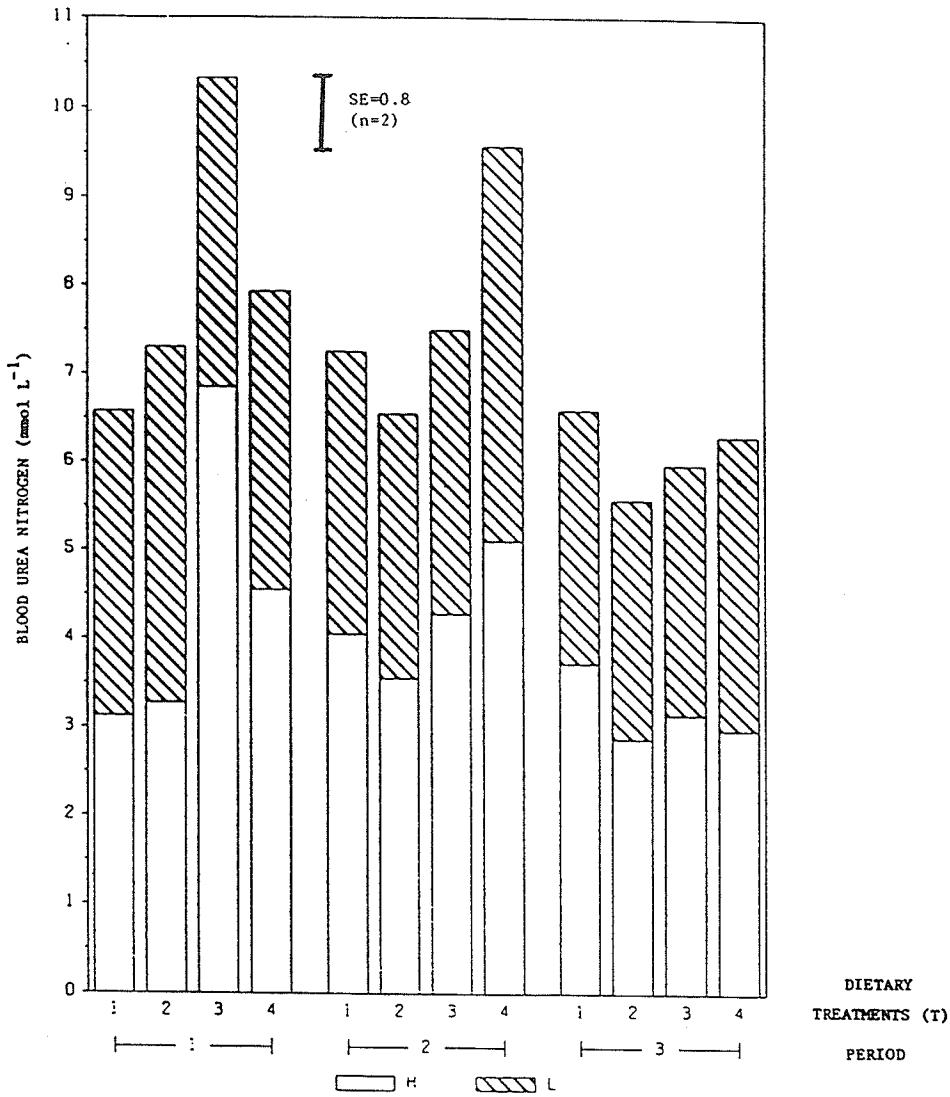


Fig. A4. Effect of four dietary treatments on means of blood urea nitrogen levels from light (L) and heavy (H) pigs in three periods - Experiment 3; SE = standard error.

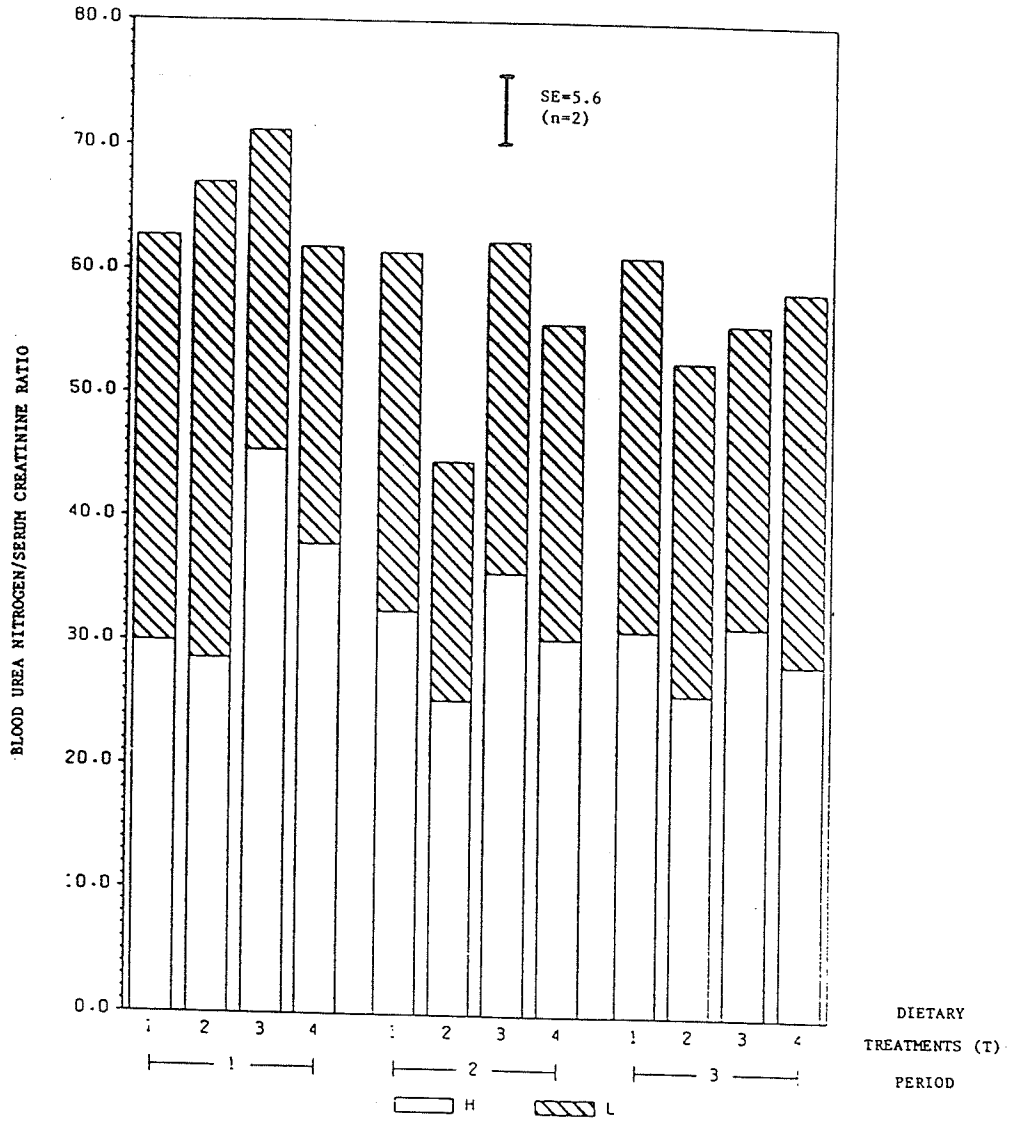


Fig. A5. Effect of four dietary treatments on means of blood urea nitrogen/serum creatinine ratios from light (L) and heavy (H) pigs in three periods - Experiment 3; SE = standard error.

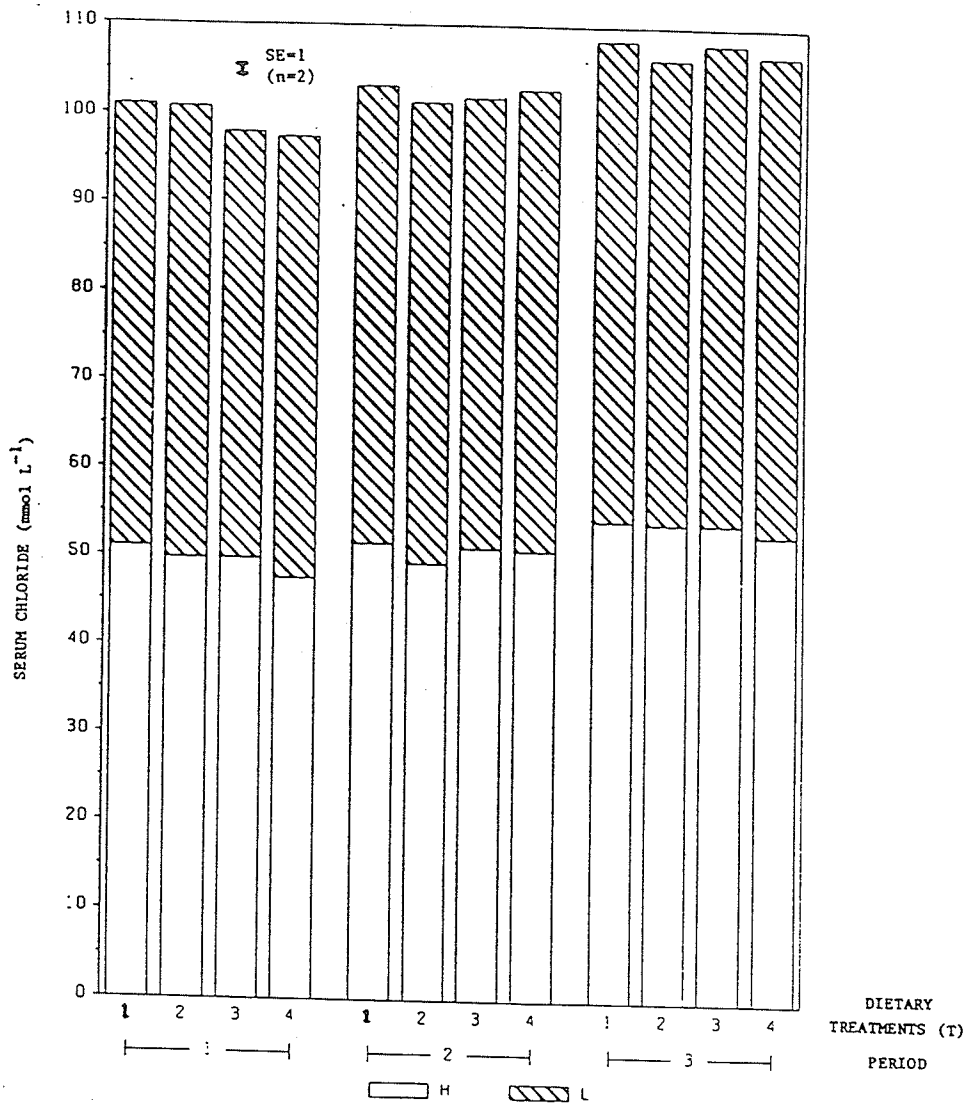


Fig. A6. Effect of four dietary treatments on serum chloride levels from light (L) and heavy (H) pigs in three periods - Experiment 3; SE = standard error.

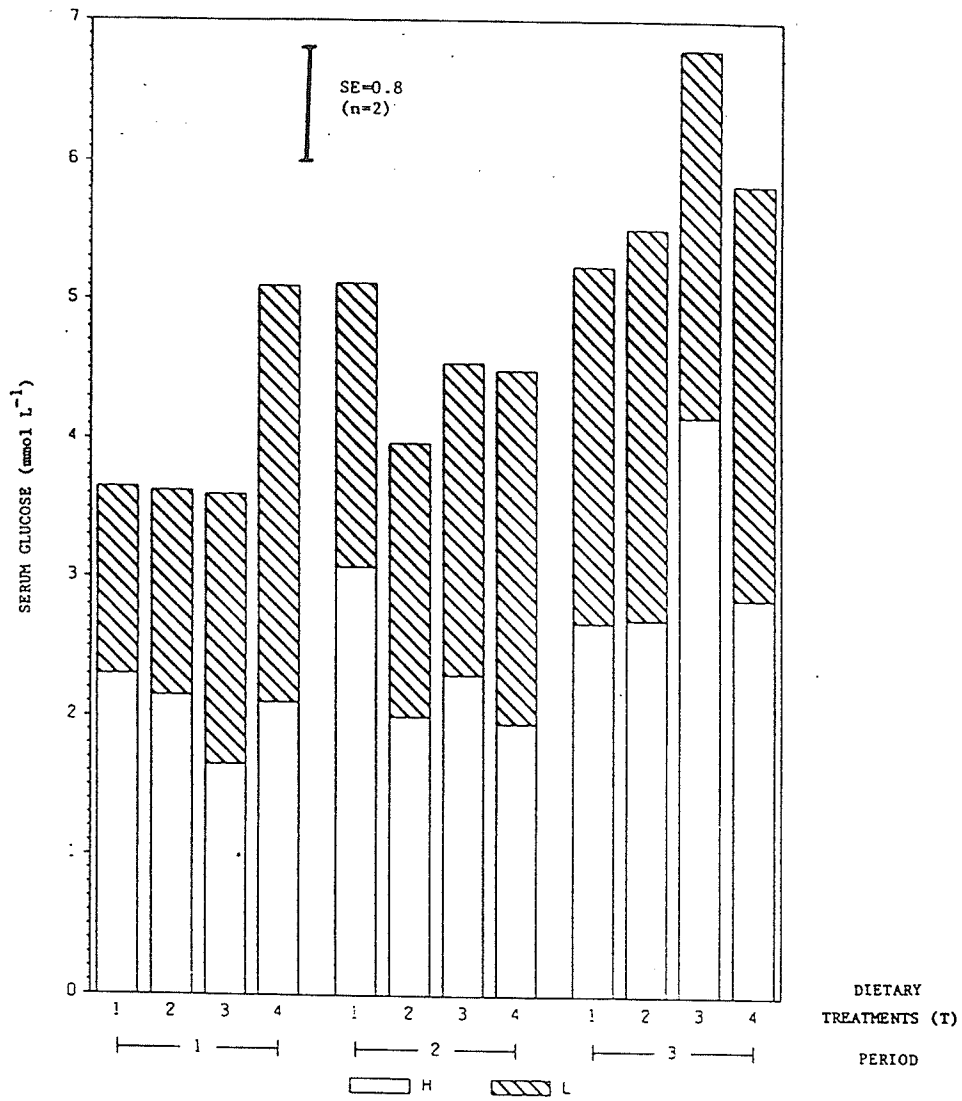


Fig. A7. Effect of four dietary treatments on serum glucose levels from light (L) and heavy (H) pigs in three periods - Experiment 3; SE = standard error.

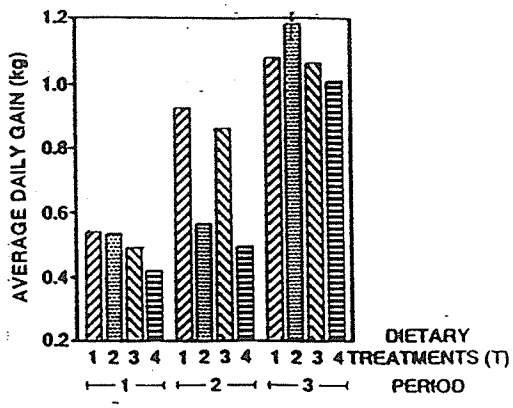
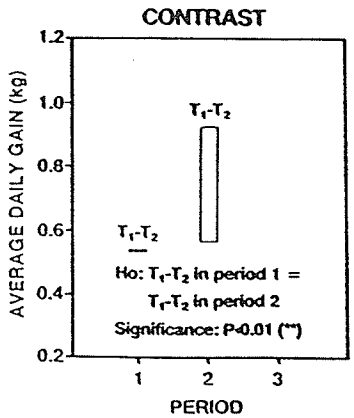


Fig. A8a.



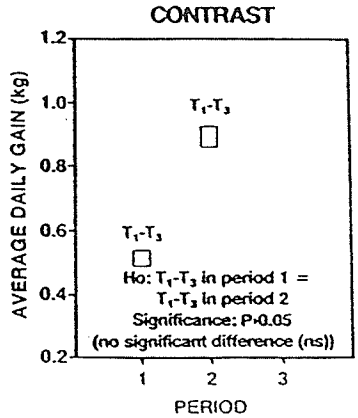


Fig. A8b.

Fig. A8c.

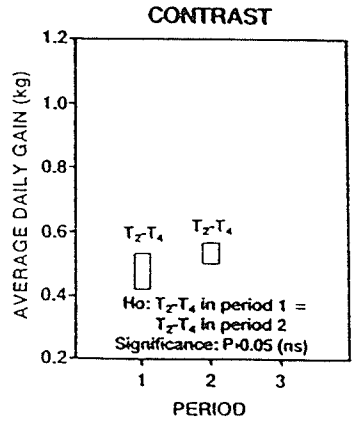


Fig. A8. Treatment differences in two periods for average daily gain (ADG) - Experiment 3. Interpretation: Fig. A8a. A high ochratoxin A (OA) level decreased ADG significantly; Fig. A8b. A low OA level did not decrease ADG significantly; Fig. A8c. No adaptation effect from a low to a hogh OA level was seen.