Acta Veterinaria Academiae Scientiarum Hungaricae, Tomus 25 (2—3), pp. 123—128 (1975)

THE FATE OF MOULD "SPORES" IN THE DIGESTIVE TRACT OF CHICKS

By

I. NYIREDY, L. ETTER, Ilona FÉSÜS and G. MAYER

National Inspectorate for Feed Control, Budapest

(Received November 26, 1973)

The importance of mycotoxicoses tends to increase with the concentration of animal raising, whereas systemic mycoses affect only certain species to a greater degree than previously when herds and flocks were smaller.

While the aetiological role of certain metabolites is more or less understood in mycotoxicoses, little is known about the pathogenesis of enteric diseases caused by hyphomycetes. Namely, the fate of conidia — endospores — ingested with the feed has been little pursued in the digestive tract, and evidence is also lacking whether these can act as direct causal factors of mycoses or mycotoxicoses.

The problem whether the orally ingested conidia and other reproductive elements of moulds survive in the digestive tract, whether they are eliminated with the faeces in a viable state, was awaiting clarification, being of immediate interest in respect of both microbiological feed control and animal health. The present experiments were performed to obtain more information on this problem.

Materials and methods

Mould strains. Mould strains frequently present in feeds and capable of causing mycosis, or mycotoxicosis, or both, were selected for the study. Although growth ability at 37 °C was regarded as the theoretical criterion of pathogenicity, strains growing only at lower temperatures were also examined. Strains growing at 37 °C: *Aspergillus candidus, A. fumigatus, A. flavus, A. niger, A. ochraceus, Circinella* sp., *Rhizopus* sp. Strains not growing at 37 °C: *Penicillium chrysogenum, P. funiculosum, P. notatum.*

The strains were propagated in 4% glucose agar containing "Stredipen a.u.v." (streptomycin bas. 500,000 IU, penicillin G—procaine 300,000 IU, K or Na salt of penicillin G, 100,000 IU) as antibiotic. Agar plates were prepared in 100-ml Erlenmeyer flasks and surface inoculation was followed by incubation for seven days at the optimal temperature. The rich fungal growth was collected by rinsing with 90 ml sterile saline per flask and "conidium"

counts in the rinsing fluid were determined by the pour-plate technique. The counts ranged between 930 thousand and 300 million, depending on the strain.

Experimental birds. A total of 100 one-day-old broiler hybrid chicks, procured from the hatchery, were used. Ten experimental groups and one control group were formed, each consisting of 10 birds. The chicks were kept in synthetic rat boxes in a well-ventilated room, at 30—32 °C temperature and appropriate air humidity. For the first 6 days, adsorbent paper, later good-quality wood shavings were used as bedding; the litter was exchanged according to requirement.

The birds were fed on a commercial chick starter until 10 days of age, after which they received a chick-rearing formula. The starter was sterilized to prevent health injury by its high germ counts. Fresh drinking water was supplied twice a day and pebbles of appropriate size were also furnished.

Fungus spores were administered at the same time every day by pipetting directly into the crop. The rinsing fluid used for this purpose was stored in the refrigerator and only the required amount was withdrawn from it each time. To prevent experimental error resulting from the deterioration or germination of the conidia in the rinsing fluid, the latter was freshly prepared every five days. Prior to administration the rinsing fluid was diluted to give a spore concentration of 1—2 million/ml. The single dose was 0.5 ml, thus each chick was given a daily dose of 0.5—1 million conidia. Administration on the above schedule was continued for 10 days, followed by a 4-day rest period and another 10-day treatment with extremely large doses, 5—40 million conidia daily, representing concentrations never occurring in feeds.

The birds were continuously examined for any change in general condition, movements, appetite, faeces (consistency, colour, odour) as well as for occasional nervous symptoms. Body weights were recorded at the beginning and on days 10, 24 and 31 of experiment.

The pathological effect of conidia was assessed by comparing body weights of treated and control birds and by postmorten examination of one chick killed in each group on days 1, 3 and 10. Rectal swabs taken from each bird between days 10 and 14 were used for mycological studies. Another chick in each group was killed on day 14. One of the six birds retained in each group for a second treatment was exsanguinated after 10 days of treatment with the extreme doses, and from the rest of the birds rectal swabs were taken on day 10, and five days later. Finally, all birds, including the controls, were killed by bleeding 31 and 32 days after beginning of the experiment. No conidium treatment was made on the day of exsanguination.

To re-isolate the fungus, streak plates were prepared on antibiotic-containing agar, on the first by a large loop from contents of crop, proventriculus, gizzard, the most anterior and most posterior segments of the small intestine,

the anterior segment of the large intestine, from coecum and rectum; later pour-plates were prepared from homogenates in antibiotic-containing agar medium of 2 cm portions of the above organs. Streak plates were also prepared with samples from trachea, lungs, air sacs, spleen, liver, kidneys and heart blood; the latter three samples were also used for culturing on Drigalski's agar. The cultures were incubated for 6 days at 26 °C for mould isolation and for 24 hrs at 37 °C for the isolation of bacteria.

To test the effect of the pH of the medium on the germination of conidia, aliquots of one strain each of *A. flavus*, *Rhizopus* and *Penicillium* were inoculated into 1% glucose broth adjusted to pH 4.0, 4.5, 5.0, 5.5, 6.0 or 7.4 and were incubated at 26 °C. The pH values of intestinal content were determined in various segments with indicator strip.

Results

Clinical observations and postmortem findings

All chicks remained healthy throughout the experiment. This can already be judged from their body weights that ranged between 700 and 1000 g at 31 or 32 days of age. The even growth rate required for reaching this weight category is in itself exclusive of the presence of mycosis, mycotoxicosis or any other disease.

Birds killed by bleeding at different times of experiment did not show any gross lesions.

Microbiological findings

Moulds related to the conidia administered could not be isolated from parenchymatous organs, blood, trachea and lungs. We usually failed to re-isolate the moulds from the digestive tract, except from the crop, of chicks below 10 days of age (Table I).

Effect of the pH of the medium on germination of conidia

With all three strains tested *(A. flavus, Rhizopus, Penicillium)*, germination failed to occur in glucose broth at pH 4.0, 4.5, 5.0 and 5.5. Both germination and mycelium formation did take place, though at a very slow rate, at pH 6.0, whereas growth was abundant at pH 7.4.

Discussion

Only part of the fungus strains tested could be re-isolated, on rare occasions, from the small and large intestines of the chicks. This supports the con-

Table I

Re-isolation of fungi from chicks killed after oral treatment with conidia for 1, 3 and 10 days

Organs	Days	Fungus strains representing the conidia used for oral infection and colony counts obtained in isolation experiments									
		A. candidus	*A. flavus*	*A. fumigat.*	*A. ochrac.*	*A. niger*	*Circinella*	*P. chrysog.*	*P. funiculos*	*P. notatum*	*Rhizopus*
Crop	1	6	9	2	—	—	6	1	2	6	—
	3	—	—	2	—	3	—	1	5	9	—
	10	—	—	—	—	—	6	—	—	—	—
Proventriculus	1	2	—	—	—	—	—	—	—	—	—
	3	—	—	—	—	—	—	—	4	—	—
	10	—	—	—	—	—	—	—	—	—	—
Gizzard	1	—	—	—	—	—	3	1	—	—	—
	3	—	—	—	—	—	—	—	—	—	—
	10	—	—	—	—	—	—	—	—	—	—
Anterior segment of small intestine	1	—	—	—	—	—	4	—	—	—	—
	3	13	—	—	—	—	—	—	—	—	—
	10	—	—	—	—	—	—	—	—	—	—
Large intestine	1	100	2	—	—	—	3	—	—	1	2
	3	98	—	—	—	—	—	—	3	4	—
	10	—	—	2	—	—	—	—	—	—	—
Coecum	1	1	—	—	—	—	2	—	—	—	—
	3	—	—	—	—	—	2	—	—	—	—
	10	—	—	—	—	—	—	—	—	—	—
Rectum (faeces)	1	—	—	—	—	—	1	1	—	—	—
	3	—	—	—	—	—	1	—	—	—	—
	10	—	—	—	—	—	—	—	—	—	—

clusion that the orally administered conidia deteriorated in the proventriculus and gizzard of both young and older chicks.

Theoretically, the deterioration of conidia and mycelia reaching the digestive tract by oral route may be due to the following reasons.

The present *in vitro* studies on pH effect and the related literary data have unequivocally shown that an inappropriate pH can be regarded as the main factor responsible for the inhibition of growth and other life functions of conidia. This accords well with the observation that pH values of 5.0—6.0,

5.0, 3.5—4.0, 5.0—5.5, 5.0 and 5.0—6.0 were measured in the contents of crop, proventriculus, gizzard, duodenum, jejunum and rectum of the birds, respectively, at 10 days of age. Since pH values are similar in the stomach and abomasum of non-ruminant and ruminant domestic mammals, respectively, there is reason to postulate that the above conclusion also applies to those animals.

Other factors acting towards the depression of life functions of ingested moulds are the intestinal O_2 and CO_2 tension which are unfavourable for moulds, and other poorly understood reactions of the macroorganism, which develop with progressing age and also depress the growth of moulds.

If germination of the ingested conidia does yet take place, owing either to a greater pH tolerance of the strain or species or to impaired functions of the host, the mycelia become digested in the stomach. Many authors have reported that the mycelia ingested with feeds massively contaminated by non-toxic fungi are undergoing a complete lysis; *e.g.*, the mycelia of *P. roqueforti*, *P. camemberti* and *Oospora camemberti* become digested in humans.

Finally, taking into consideration that re-isolation of the experimentally administered fungus strains was successful only in two cases, the conclusion lies close at hand that the walls of the ingested conidia become lysed by the digestive juices.

The present findings permit some important conclusions in respect of poultry health, which seem to apply to other useful animal species as well.

In view of the practically complete destruction of conidia and other reproductive structures of moulds in the alimentary tract, oral ingestion of even very large amounts of such fungal stages involves practically no risk of mycosis or mycotoxicosis. Accordingly, no mycotoxicosis develops unless the ingested feed itself contains mycotoxins in a toxic concentration, and even the fungi growing readily at body temperature do not cause mycosis unless the mycelia present in the massively contaminated feed do not become digested by the digestive juices, *viz.*, if the conditions prevailing in the alimentary tract are such as to not inhibit the germination of conidia, further development of the ingested mycelia or their access to the blood and lymph circulation. Such conditions, however, occur only in animals with impaired health, lowered powers of resistance, or disorders in the secretion of digestive enzymes and bile, *viz.*, if the gastric mucosa is mechanically impaired by feed particles, bacterium-induced ulceration, *etc.* Mycoses always have a multifactorial aetiology, depending greatly on individual predisposition. They preferably occur in young animals and in animals with deranged constitution and consequent depressed metabolic activity, owing to unnatural keeping conditions. Fungus strains not growing at body temperature are never responsible for mycosis.

Moulds responsible for lung mycosis never cause disease on ingestion with the feed. Chicks infected in the present study from 2 days of age over

20 days with 0.5 to 40 million conidia administered directly into the crop developed neither symptoms nor gross lesions of lung mycosis. The realistic evaluation of microbiological feed analysis, the improvement of the outlook of breeders as well as aspects of guarantee equally demand that it should be emphasized over and over again that the fungus species most frequently responsible for lung mycosis, *viz.*, *A. fumigatus*, *Mucor* species, *etc.*, are ubiquitous and are pathogenic on inhalation only, never on oral ingestion. It follows that isolation from a given feed of *A. fumigatus* or some other fungus pathogenic for the lung is no proof of the responsibility of the fungus for lung mycosis, except if the plaintiff can present authorized evidence that the fungus responsible for the pneumomycosis had not been present in the unit, flock or herd prior to the introduction of the suspect feed or that the onset of the disease was closely associated with the feeding of this diet. Evidence to the first criterion is practically impossible, considering the omnipresence of the incriminated fungi, and fulfilment of the second criterion is far from being easy, since the disease is known to be multifactorial. Related studies in this laboratory also support the conclusion that the fungal contamination of commercial compound feeds is practically never of such a degree as could cause disease in poultry.

Summary

Large doses of spores of various mould species were administered directly into the crop of day-old chicks to obtain evidence whether oral ingestion of the reproductive stages (conidia, endospores) of moulds can cause mycosis or mycotoxicosis in this species. Seven species growing well at the body temperature of the birds and three species not growing at that temperature were tested. Individual daily doses of 500,000 to 1,000,000 conidia were administered for 10 days and after a subsequent 4-day rest period the daily dose was risen to 5—40 million conidia, given for another 10 days. Part of the species could be re-isolated from the digestive tract, above all from the crop, for 3 days, exceptionally for 10 days. Isolation from visceral organs and blood failed throughout. This supports the conclusion that oral ingestion of conidia cannot in itself elicit either mycosis or mycotoxicosis.

Address of the senior author: Dr. István NYIREDY, 1085 Budapest, József krt. 38, Hungary.