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Impact of sorghum ergot in layer hens

A report for the Australian Egg Corporation Limited and the Grains Research and Development Council

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and

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Forward

Sorghum is one of the most important grains fed to poultry. However, sorghum can be contaminated by a fungi, sorghum ergot, that produces the toxin dihydroergosine. The presence of ergot contamination in some Australian sorghum crops has resulted in the poultry industries losing some confidence in the use of this grain in poultry feeds.

Earlier research has suggested that poultry tolerate higher levels of ergot toxin in feedstuffs than do ruminants, horses or pigs. Furthermore, laying hens seem to be more tolerant than young chicks.

Recently, a limit of 0.3% sorghum ergot has been inserted in Queensland Stockfeed Regulations.

As a consequence, the Grains Research and Development Corporation (GRDC) and AECL (formerly RIRDC Egg Program) funded this research project to determine the safe practical limits for use of ergot contaminated sorghum grain in layer diets.

Experiments were conducted to investigate the effects on egg production and feed digestibility of feeding ergot contaminated sorghum grain to laying hens and to determine if a commercially available toxin binding feed additive had any impact on the effects of the ergot. An experiment was also conducted to determine whether any toxin residues were transferred to the eggs.

The results of these experiments indicate that very high levels of ergot contamination may cause small reductions in layer productivity but do not result in any toxic residues in eggs. The researchers conclude that the safe limit for ergot in feed for laying hens could be raised from 0.3% to at least 1% without significantly increasing risk to layer health or productivity or to the food safety of eggs.

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Executive Summary

Three experiments were conducted.

The first experiment was conducted to determine the effect of sorghum ergot (Claviceps africana) alkaloid (dihydroergosine, DHES) and Mycosorb® binding agent on the production of laying hens. A total of 96 commercial ISA Brown laying hens were paired caged in a semi-controlled environment room, and fed different levels of ergot contaminated sorghum. The basal diets were :(1) 24 mgkg-1 DHES, (2) 12 mgkg-1 DHES, (3) 6 mgkg-1 DHES and (4) zero DHES (normal sorghum). Mycosorb was added to half of each basal diet, making 8 diets in total. The diets were fed and egg production, feed intake and egg weight were measured, and feed conversion ratio (FCR) was calculated for a period of 6 weeks. Egg production from the diet containing 24 mgkg-1 DHES was significantly less than that produced from the other diets. The addition of Mycosorb was beneficial. Egg weight at week four was significantly decreased by 24 mgkg-1 DHES. Egg mass decreased as the level of DHES in the diets increased but Mycosorb addition increased egg mass. Neither DHES nor Mycosorb had any significant effect on feed intake or feed conversion ratio overall. This work shows that if the maximum limit of sorghum ergot was increased from the present level of 0.3% to 1% (i.e. from approximately 1 mgkg-1 to 5 mgkg-1 DHES) it would not significantly affect the production or efficiency of laying hens.

The second experiment was conducted to determine the effect of DHES on dry matter digestibility in laying hens. A total of 32 commercial Brown laying hens 66 weeks old were pair caged in a semi-controlled environment room, and fed different levels of ergot contaminated sorghum. The diets were formulated to contain :(1) 24 $mgkg^{-1}$ DHES, (2) 12 $mgkg^{-1}$ DHES, (3) 6 $mgkg^{-1}$ DHES and (4) zero DHES or normal sorghum. The calculated nutrient content of the diets was estimated to be adequate for good production for ISA Brown hens. Mycosorb® was added to half of each diet, making 8 diets in total. The diets were fed and total faeces were collected from two replicate 2-bird cages per treatment for a period of 24 hours. Representative samples of all diets and faecal collections were analyzed for dry matter content and the apparent dry matter digestibility of each of the eight diets was calculated. There was no significant ($P>0.05$) effect of DHES or Mycosorb addition on dry matter digestibility in laying hens. However, there was a trend of decreasing digestibility with increasing level of DHES in the diet. However it was concluded that increasing the maximum allowable concentration of ergot in layer diets from 0.3% to 1% (from 1 to 5 mg DHES/kg diet) would not significantly affect the digestibility of the diet.

The third experiment was conducted to investigate whether feeding sorghum ergot to hens produces alkaloid residues in eggs. Hens were fed diets containing up to 24 mg DHES/kg for several weeks and eggs were collected daily. Over 80 eggs from ergot-fed birds and 80 from control birds were blended and assayed by an ELISA that is very specific for DHES. The ELISA had a detection limit of 0.005 mg/kg DHES, but DHES was not detected in any egg. Over 40 eggs from the ergot-fed birds were also assayed by HPLC with fluorescence detection, also with negative results (<0.02 mg DHES/kg). On average, only 29% of ingested DHES was recovered in the excreta, suggesting that DHES was rapidly degraded in the intestine. The regulatory limit for ergot in feed for laying hens might be raised from 0.3% to 1% (about 1 mg DHES/kg to about 5 mg DHES/kg) without significantly increasing the risk of adverse production effects or residues in eggs.

1. Literature review - The effect of sorghum ergot (*Claviceps africana*) toxicity and its control in poultry

1.1. Sorghum ergot

1.1.1. General introduction

Sorghum (Sorghum bicolor) is one of the most important grains fed to poultry. The presence of ergot contamination means that the poultry industry has lost some confidence in the use of this grain in poultry feed.

Sorghum is well known as a summer growing grass native to Africa and Asia, and is produced in large quantities throughout the USA, China, India and Africa, where it is used for both human and animal consumption. It has also been grown in Australia (QLD & NSW) since the end of World War II, where it is marketed primarily as a stockfeed.

Sorghum develops several diseases, one of which is the fungal disease sorghum ergot (SE). It produces mycotoxins that pose a threat to both humans and animals when consumed. In Australia sorghum ergot (Claviceps africana) was identified in 1996 (Bandyopadhyay *et al*.1996).

1.1.2. Mycotoxins

Of the known 200,000 species of mould, about 100 have been shown to produce toxic compounds. These moulds produce chemical compounds known as mycotoxins, which are poisonous when eaten. They can be present in both grains and forages, and are commonly found in some crops growing in the field or during storage, and the contamination is a serious problem for agricultural industries. Contaminated products may produce undesirable effects in animals or humans consuming them. The poisoning effects of the mycotoxins are very serious. The most important group of fungi, from the point of view of prevalence in grains and mycotoxin contamination, are the *Claviceps* species which produce ergot alkaloids (Maryam, 1999).

It is estimated that approximately 25% of the world's cereals are contaminated with known mycotoxins (Devegowda *et al*, 1998), while a higher percentage could be contaminated with unidentified mycotoxins. Sorghum ergot alkaloid (SEA), a recently identified mycotoxin in Australia (Blaney, 1996), has caused significant depression in growth, poor FCR, a reduction in dietary ME and increased diarrhoea and mortality preceded by apparent gasping for breath but no difference in feed intake in chickens (Mannion and Blaney, 1998). The main alkaloid (dihydroergosine, DHES) in SE has been reported to be much less toxic to laboratory animals than rye ergot alkaloid (Frederickson *et al.,* 1991).

Mycotoxin contamination of human food and livestock feeds has come to the attention of the World Health Organisation (WHO) due to global concern in relation to human and animal health. Although limits for mycotoxin contamination can be established, these are difficult to achieve in practice, and contamination may not be avoidable without unacceptable losses of food and feeds (Maryam, 1999).

1.1.3. Mycotoxin production

Mycotoxins are produced when conditions of high relative humidity (100% optimum) and mean temperatures of 19 – 21 °C favour the growth of the fungi. These develop more abundantly during wet seasons. Different toxin-producing moulds may be present in an ingredient or feed at various concentrations. Under ideal growing conditions these moulds may produce toxins in only minute quantities but their effects can be huge.

1.1.4. Ergot alkaloids

The term ergot is a common name given to the sclerotia (fruiting bodies) of species of *Claviceps* (Mantle, 1975) naturally occurring in rye, wheat, barley, sorghum and some related crops.

Rye ergotism, also known as "St. Anthony's Fire", was one of the first mycotoxicoses to be recognised in the world (van Rensburg and Altenkirk, 1974). Ergot alkaloids that have been responsible for cases of human poisoning can be traced to a known specific class of mycotoxin from the historic record. Animal feeds, especially rye, are often contaminated with ergot alkaloids from Claviceps, which is entophytic in pasture grasses worldwide.

Ergot alkaloids are common in grain products but at levels that probably have minimal health risk to humans. However they do pose a significant health risk to animals. The signs produced in animals ingesting ergot alkaloids typically include poor weight gain, poor milk production in lactating animals, various reproductive disorders and in extreme cases, the classical gangrenous loss of extremities such as feet, tails and ears.

1.1.5. Characteristics of sorghum ergot (*Claviceps africana*).

The sclerotia of *Clavicepes africana* are oval or spherical parasitic structures bearing a small distal sphacelial cap, measuring 4-6 mm by 2-3 mm. They each have a white plectenchymatous medulla bounded by a thin red-brown cortex, appearing patchily flecked red and covered by adherent sphacelial fructification.

The stomata initially appear as a pale, globose proliferation of the sclerotium at one or two places from which up to 5 to 6 stomata arise. Fully extended stipes (8-15 mm by 0.3-0.6) are pigmented purple, adjacent to the capitulum. Capitula (0.5-1.3 mm) are sub-globose and intensely purple. Perithecia measure 86-135 µm by 123-126 µm with mature asci in situ measuring 140µm by 3-4µm containing eight ascospores each up to 45µm by 0.8-1.2 µm (Frederickson *et al*., 1991; Bandyopadhyay *et al*., 1996; Bandyopadhyay *et al*., 1998).The sphacelial stage of asexual fruitification of Claviceps africana is a highly convoluted, white parasitic body, 5-8 mm long, bearing in discrete pockets the hyaline, mononucleate, oblong to oval macroconidia of 9-17 µm by 5-8 µm, slightly constricted at the centre and with two polar vacuoles and spherical microconidia 2-3 µm in diameter. Pear-shaped secondary conidia borne on sterigma-like processes are 8-14µm by 4-6.5 µm with a distinct hilus (Frederickson *et al*., 1991).

Secondary conidiogenesis is solitary because of the rapidity and the manner in which it occurs in the honeydew (Frederickson *et al*., 1989; Bandyopadhyay *et al*., 1990). Formation of secondary conidia results from iterative germination of macroconidia on the surface of thin honeydew when relative humidity is high. Due to high osmotic potential generated by the sugars in the honeydew matrix (or because of the inhibitory nature of the sugars), the macroconidia inside the viscous honeydew do not germinate (Mantle *et al*., 1997). However, being hygroscopic, the honeydew surface absorbs water from rain or dew (atmosphere), lowering its osmotic potential (or dilutes the inhibitory sugars) and renders the honeydew thin. As a result, macroconidial germination is iterative and involves the extension of germ tubes outside the honeydew surface. These tubes are functionally conidiophores terminating in apical secondary conidia that are easily detachable and disseminated by wind (Bandyopadhyay *et al*., 1996). Moreover, macroconidia in the outer layer of the honeydew germinate to produce thick, branched or unbranched germ tubes that enmesh to form a firm hyphal mat that provides a stable surface on the otherwise fluid honeydew.

1.1.6. Life cycle of *Claviceps africana*

The path involved in the infection by *Claviceps africana* is similar to that of *Claviceps sorghi* and is described in detail by Federickson (1990) and Bandyopadyay *et al*. (1998). The initial spread of primary infection in the field is possibly established by ascospores from germinating sclerotia, sphacelial conidia and conidia from collateral grass weed hosts. The sclerotial germination could probably be by sexual means. Although the thick-walled sclerotia protect macroconidia contained within its locules from edaphic elements, the sclerotial wall slowly degrades with the onset of rain or insect attack, releasing macroconidia on the soil surface, the macroconidia germinating on moist soil to produce secondary conidia. The secondary conidia become wind-borne and cause new infections both near and far from the initial infection.

1.2. Toxicity of ergot alkaloids

1.2.1. Common toxicity of ergot

One of the foremost issues of ergot infection is the risk associated with the toxicity of alkaloids produced in the sclerotia. The severity of the problem in livestock and poultry depends on factors such as species, age and general health status of the animal, period of exposure, type of ergot and amount consumed. The toxic alkaloid content of ergot-contaminated grains and grasses can depend on the strain and stage of maturity of the fungus, type of host plant, growing condition and geographic conditions. The total concentration and composition of the ergot toxins may be highly variable between individual sclerotia and consequently throughout a contaminated field. Exposure to the large variety of ergot toxins is manifested by a wide range of chemical signs (Prelusky *et al*., 1994).

When ingested, the alkaloids result in various peripheral and central physiological actions (Gilman *et al*., 1985). The peripheral effects involve contraction of smooth muscles including those of blood vessels. This results in the occlusion of blood vessels which gives rise to “gangrenous ergotism”. The affected organ, for instance leg, becomes swollen and inflamed, loses sensation and turns black (necrosis, gangrene). Moreover, ergot alkaloids also induce uterine contractions and reduce postpartum haemorrhage. It is only at term that the uterine muscle is more sensitive than other smooth muscles to ergot and therefore, these alkaloids can not be used to induce abortion. In humans, the antiserotonin effect of rye ergot alkaloid (ergotamine tartrate) contributes to the efficacy of these compounds in relieving migraine (Gilman *et al*., 1985).

Generally, alkaloids of rye ergot restrict the blood supply to the extremities, causing lameness and gangrene. The alkaloids of sorghum ergot are much less active in this particular respect and blood supply effects have not been previously seen in field cases but were reported in experimental feeding trials by Deo (2000). Manev *et al*. (1989) and Frederickson *et al*. (1991) reported that the alkaloid in sorghum ergot (dihydroergosine) was less toxic than the rye ergot alkaloids. But despite reports from overseas, sorghum ergot in Queensland, Australia, appears to be just as active as rye ergot in reducing the weight gain of chickens (Mannion and Blaney 1998) and in reducing the milk production of sows and dairy cows (Blaney *et al*., 1997; Blaney *et al*., 1998).

1.2.2. Common effects of ergot on animal performance

In the livestock industry, mycotoxin contamination is a serious economic problem because it prevents animals from reaching their optimum body weight gain, feed efficiency and reproduction. The symptoms of mycotoxicosis range from reduced feed intake to swelling of mammary glands, kidney and liver damage, and nervous system disorders. The adverse effect of mycotoxin on animal performance may be only part of the problem. An equally serious issue is that some alkaloids may be transferred from the feed through the animal and enter the human food chain through meat, dairy products and eggs. Consumers who are now more aware of food-borne health risks such as mycotoxin contamination are becoming more vocal about their rights to a safe food supply.

Ergot is toxic to animals. Animals consume ergot by eating the sclerotia present in contaminated feed. All domestic animals are susceptible, including poultry. Cattle seem to be the most susceptible. Two known forms of ergotism exist in animals, an acute form characterised by convulsion, and a chronic form characterised by gangrene (McMullen and Stoltenow, 1998). A third form of ergotism is characterized by hypothermia (decreased body temperature) in cattle, and a fourth form is characterized by agalactia (no milk) and lack of mammary gland development, prolonged gestation and early foetal death.

The responses of animals consuming ergot are usually quite variable and are dependant on variations in alkaloid content, frequency of ingesting ergot, quantity of ergot ingested, climatic conditions under which ergot grow, the species of ergot involved and the influence of other impurities in the feed.

The most common effects on livestock include:

- Behavioural effects – lameness, incoordination, convulsions, and difficulty in breathing, excessive salivation and diarrhoea.
- Dry gangrene of the extremities.
- Reproductive effects – abortion, high neonatal mortality and reduced lactation.
- Digestive effects – reduction in feed intake, weight gain and feed conversion efficiency (Cheeke, 1998).

The ergot alkaloids have been responsible for cases of human toxicosis since the prehistoric times and rye ergot alkaloid is certainly one of the few for which human poisoning can be traced to a known specific class of mycotoxin from the historic record. Rye ergotism, also known as St. Anthony's Fire (or Holy Fire), was one of the first mycotoxicoses to be recognised in the world. Ergotism is rare now because of the strict guidelines for allowable ergot bodies in grain but can still be found in livestock fed poor quality grain (van Rensburg and Altenkirk, 1974).

1.2.3. The effect on poultry production

The presence of ergot alkaloids either in ruminant or non ruminant animal rations may have significant adverse effects on overall performance. Poultry appear to tolerate higher levels of these toxins in feedstuffs than do ruminants, horses or swine. Characteristic signs of increased ergot in broilers are reduced feed consumption, depressed growth, incoordination, poor feathering and vasoconstriction resulting in elevated blood pressure, restricted blood flow and subsequent necrosis of toes, beak and skin (Young and Marquardt, 1982 ; Rotter *et al.,* 1985a,b). Similar results were found by Mannion and Blaney (1998) and Deo (2000) where poultry showed depression in growth and poor feed conversion ratio when fed ergot contaminated sorghum in Queensland.

Higher dietary ergot levels (0.4 to 9.0 %) resulted in a depression in growth and increased chick mortality. Laying hens were shown to be more tolerant to dietary ergot (9%) than chicks, but egg production was adversely affected at higher levels of ergot alkaloids (Bandyopadhyay *et al.,* 1998). Queensland Stockfeed Regulations limit ergot in stockfeed to 0.02%, which covers all varieties of ergot. Recently a limit of 0.3 % sorghum ergot has been inserted in Queensland Stockfeed Regulations, which was effective from 1st October 1997 (Blaney *et al*,1997).

Sorghum ergot contains a primary alkaloid known as dihydroergosine (DHES). DHES was recently identified as the main alkaloid present in sorghum ergot in Australia (Blaney, 1996). The discovery and negative impact of sorghum ergot alkaloids (SEA) in poultry have been recently reported in the USA (Bailey and Fazzino, 1998) and in Australia (Blaney *et al*; 1998). Sorghum ergot alkaloid has significant adverse effects on overall performance. It has caused significant depression in growth, poor FCR, a reduction in dietary ME and increased diarrhoea and death preceded by apparent gasping for breath but no difference in feed intake in hens.

In studies done by Rotter and co-workers (Rotter *et al*., 1985 b), they reported that in growing chicks, a concentration of 3.1 $mgkg^{-1}$ total rye ergot alkaloid produced a statistically significant reduction in weight gain, and feed efficiency, progressing to an 80% decline in weight when fed 24.6 $mgkg^{-1}$ ergot alkaloids. At lower levels (<10$mgkg^{-1}$ ergot alkaloids) no signs of ergot poisoning other than decreased feed performance and lack of energy were noted. It was also noted that above 12$mgkg^{-1}$ rye ergot alkaloids there was a significant and progressive increase in mortality and only those birds that died showed lesions of peripheral necrosis.

O'Neil and Rae (1965) indicated the tolerance level for chicks was 30$mgkg^{-1}$ of rye ergots (about 7.0$mgkg^{-1}$ ergot alkaloids) in the diet, with respect to egg production, feed consumption and body weight maintenance. Egg quality and hatchability were not affected at this level. Bailey and Fazzino, (1998) have shown a significant increase in organ (liver) weight in broilers fed SE for three weeks, suggesting possible hepatotoxicity.

Mannion and Bailey (1998) investigated the effects of sorghum ergot (*C. africana*) on broiler chickens. They fed ergot contaminated sorghum feeds to broilers and attempted to reduce the difference in nutrient composition between the ergot-contaminated sorghum and uncontaminated sorghum. They found that there was a slight reduction in growth and performance of the broilers fed ergot. However, it was unclear whether the reductions were specifically caused by the ergot or by the difference in nutrient content. In spite of this, poultry appear to tolerate higher levels of these toxins in feedstuffs than do ruminants, horses or pigs (Mannion and Blaney, 1998).

1.3. Control of sorghum ergot alkaloid (SEA)

A major challenge presented by mycotoxin contamination of livestock feed is the fact that mycotoxins are stable compounds and are not easily removed from finished feeds. However, once ergot contamination is confirmed, the livestock producer or feed manufacturer must select a procedure to reduce or eliminate its effects.

After the identification and recognition of mycotoxicosis, several strategies are used to minimize the adverse effects of mycotoxins on livestock and to prevent human exposure. Various practices have been used to control ergot alkaloids including the use of dietary supplements, prevention through genetic selection and detoxification of feeds and feedstuffs by physical, chemical or biological methods. Pre treatment methods (such as use of fungicides, seed treatment by NaCl and mechanical separation of fungal affected feed) have been developed to control ergot fungi in grain cereals to reduce toxicity.

Physical methods include cleaning and washing, dehulling, polishing, separation of contaminated from non-contaminated kernels, a screening technique, organic solvent extraction, heat treatments, irradiation and pH changes. The success of these procedures depends to a large extent on the initial degree of contamination and on the distribution of mycotoxins in the feed (Deo, 2000).

1.3.1. Breeding sorghum for resistance

Breeding for ergot resistance is based on the tolerance of sorghum germplasm to ergot in unfavourable weather conditions during early flowering. The reaction of sorghum to ergot caused by Claviceps africana (Frederickson *et al*., 1991) is dependent on weather conditions during early flowering. As a result a suggested method of screening for resistance is to plant sorghum so as to synchronise flowering and inoculation with unfavourable disease conditions.

Moreover, variations in ergot incidence and severity in sorghum lines is related to tolerance of lines to ergot favourable conditions such as pre-flowering cold stress and the ability to escape infection by ensuring effective and rapid pollination and fertilization (Bandyopadhyay *et al*, 1996). However, ergot

resistance is limited and it is important to recognize that ergot control is not limited to a single control strategy but requires integration of many technologies (Frederickson *et al*, 1993).

1.3.2. Grain screening technique

Rapid screening methods are now available for the detection of mycotoxins. Grains contaminated with mycotoxins are exposed to ultraviolet illumination for examination of the presence of various moulds in damaged grains. Rapid minicolumn chemical tests are now available that can detect specified levels of mycotoxin in various materials. In diagnostic laboratories in tropical countries, test kits that can be used to detect a range of mycotoxins using thin layer chromatography (TLC) for quality control tests of ingredients (Gimeno, 1979).

1.3.3. Heat treatment

Some mycotoxins are very resistant to thermal inactivation and therefore the procedures based on detoxification by heating (boiling water, autoclaving, roasting baking) usually result in little change in mycotoxin levels (Park and Liang, 1993). The effect of heat treatment under various pressures for various lengths of time on the destruction of mycotoxins produced by the different ergot species isolated from grains and grown on yeast medium was investigated by Hassanin *et al*., (1993). Studies done by Deep and co-workers (Deep *et al*., 1992) showed that heat treatment caused destruction of aflatoxin M1 (AFM1) in buffalo milk and the rate of destruction was related to the time and temperature of the heat treatment applied.

1.3.4. Adding binding agents

Attempts have been made to reduce the adverse effect of toxins in animal feeds by adding binding agents. The use of binding agents has shown positive results by removing or diminishing the adverse effects of mycotoxins in poultry feeds.

One of the most recent supplements used to reduce the impact of mycotoxin contaminated feed is a glucomannan yeast extract (Mycosorb®, Alltech) (Devegowda, 1996). Mycosorb® is a functional carbohydrate extracted from the yeast cell wall. Mycosorb® at a low rate of inclusion (0.1- 0.2 g/kg), adsorbs several important mycotoxins at a high level, and does not bind vitamins and minerals (Anonymous, 2000).

Research has found that Mycosorb improved the growth and feed efficiency of broilers fed diets contaminated with aflatoxin, ochratoxin, and T-2 toxin. Mycosorb was also shown to be effective against sorghum ergot alkaloid. In several feeding trials, Mycosorb was cost effective in terms of restoring lost performance (Blaney *et al*., 1998, Deo, 2000).

A number of products claim to reduce the activity of mycotoxins in animal feeds. Some bentonites and other aluminosilicate clays bind mycotoxins in the intestine thereby preventing their absorption. Some researchers have found them to be effective both in vitro and in vivo. Clays bind to mycotoxins through electrical charges; however, not all mycotoxins have electrical charges.

Although the mineral clay binders satisfy some of the criteria for successful mycotoxin reduction, there are some shortcomings. They only bind a narrow spectrum of toxins and offer little or no protection against toxins such as zearalenone or the tricothecenes and they have relatively high inclusion rates, generally more than 1% of the diet.

Field studies have shown that Mycosorb® improved the growth and feed efficiency of broilers fed diets contaminated with aflatoxin, ochratoxin, and T-2 toxin (Deo, 1996). Mycosorb® was also shown to be effective against sorghum ergot alkaloid. In several feeding trials, Mycosorb® was cost effective in terms of restoring lost performance (Blaney *et al*, 1998).

Mycosorb® is a binding agent supplied by Alltech Ltd. Kentucky, USA.

In broilers, only a 0.02-0.03-unit improvement in the feed conversion ratio is needed for Mycosorb® to be profitable. In laying hens, depending on egg price, an improvement in egg yield of approximately 1% is about "breakeven" for cost. So improvements over 1-2% in rate of lay, or a similar reduction in cracked eggs are sufficient to be profitable. In breeder hens, where the value of a day old chick comes into play, increases in hatchability of the order of 1% are generally profitable. Based on this evidence, Mycosorb® may fulfill the necessary criteria for a safe and cost effective control for mycotoxin contamination in animal ingredients and feed (Bruerton, 2001).

1.4. Conclusion

Sorghum ergot (*Claviceps africana*) contains a primary alkaloid known as dihydroergosine, DHES. DHES has a negative impact on the performance of livestock. However, poultry appear to be more resistant to ergot alkaloids than other livestock. High levels of DHES concentration in poultry rations have caused reduced feed intake, depressed growth, incoordination, poor feathering and vasoconstriction resulting in elevated blood pressure and increased diarrhoea. The addition of a binding agent (Mycosorb) improved the performance of meat chickens.

This review concludes that sorghum ergot is a risk to both the sorghum and livestock industries. The effects of feeding sorghum ergot alkaloid on the production of laying hens and on the excretion of DHES into eggs need to be investigated, because it is possible that ergot contaminated sorghum could best be fed to poultry rather than to other livestock.

2. The effect of sorghum ergot (*Claviceps africana)* Alkaloid and Mycosorb® Binding Agent on the production of laying hens

2.1. Objectives

The objective of this study was to determine the effect of sorghum ergot alkaloid (SEA) with or without Mycosorb binding agent on the production of laying hens. It is important to examine the effect of ergot alkaloid and Mycosorb binding agent on the production of laying hens, especially on egg production, egg weight, egg mass, feed intake and feed conversion ratio, so that the risk of feeding contaminated sorghum can be assessed, by both the grain and egg industry.

2.2. Materials and methods

2.2.1. Birds

A total of 96 commercial ISA Brown laying hens 60 weeks old was used in this experiment at the University of Queensland Gatton Campus. The hens were fed a standard layer diet for four days before changing to the experimental diets. The results of the six weeks of feeding the experimental diets are reported.

2.2.2. Housing

The birds were housed in a semi-controlled room with an average temperature of 22°C. Dry and wet bulb mercury thermometers were used to measure maximum temperature and humidity. Maximum temperature ranged between 20 and 27°C and maximum humidity ranged between 52 and 76%. Ranges of temperature and humidity in which birds perform normally are 17-26°C and 40-75% RH. In this experiment high RH coincided with periods of low maximum temperature except during week 2 (Table 1). A small fan operated continuously to ventilate the room, which had open louvres on the southern side. Fluorescent light was controlled by a time clock to provide 16 hours light and 8 hours of dark.

Table 1. Temperature and relative humidity recorded during experiment.

Week	1	2	3	4	5	6
Max T°C	27	27	23	24	23	20
Max RH%	52	76	76	67	68	74

2.2.3. Water supply

Drinking water was provided to the hens *ad libitum* via nipple lines attached to the mains water supply line via a pressure reduction valve.

2.2.4. Experimental feed

The basal layer diet was formulated using sorghum, fishmeal, vegetable oil, limestone, vitamin & mineral mix, DL-Methionine and L-Lysine (UFFF, 1986).

Ergot contaminated sorghum analyzed to contain 32 $mgkg^{-1}$ alkaloid was used as 76 % of diet 1, and normal sorghum was used to dilute the ergot in each successive diet. The normal sorghum had a lower crude protein level than the ergot contaminated sorghum (89 cf 147 gkg^{-1} CP) so soybean meal was added to equalize the CP levels of the diets. This meant that basal diet 1 was calculated to contain 24 $mgkg^{-1}$ DHES, diet 2 to contain 12 $mgkg^{-1}$ DHES, diet 3 to contain 6 $mgkg^{-1}$ DHES, and the control diet contained normal sorghum only.

The ingredient composition and nutrient analysis of the experimental diets are shown in tables 1 and 2. These four basal diets were fed with or without a commercial esterified glucomannan (EGM) based mycotoxin binder (Mycosorb® B/N 195825 Alltech, Inc) manufactured from yeast by-products. The diets were weighed into individual feed troughs for each pen of two birds. Top up weights were recorded and feed residues weighed and discarded each week.

Table 2. The ingredient composition of the experimental diets ($glkg^{-1}$)

Ingredient	Diet 1	Diet 2	Diet 3	Diet 4
Sorghum (ergot) (14.7 % CP)	754.6	354.5	169.6	0
Sorghum (Non-ergot) (8.9 % CP)	0	354.5	509.0	655.8
Soybean Meal (49% CP)	0	60	100	130
Vegetable Oil	20	18.6	17.8	17.2
Limestone	90	83.9	80.3	77.6
Vit & Min Premix*	2.0	2.0	2.0	2.0
Choline	2.0	2.0	2.0	2.0
DL-Methionine	2.0	2.0	2.0	2.0
L-Lysine	1.0	1.0	1.0	1.0
Yolk pigment	2.0	2.0	2.0	2.0

* The Vitamin and Mineral Premixes added the following (in mg/kg) to the diet: 39.2 IU D_3, 15 IU Vit A, 4.2 IU Vit E, 1.74 IU Vit K, 126.7 mg Mn, 100.3 mg Zn, 415,9 mg Fe, 10.7 mg Cu, 0.14 mg S, 018 mg Se, 0.11 mg Co, 4.1 mg thiamine, 7.7 mg riboflavin, 50.4 mg niacin, 3.5 mg pyridoxine, 8.1 mg panthothenate, 0.19 mg folic acid, 11.31 IU choline.

Table 3. The ration analysis (calculated nutrient content of basal diet, gkg^{-1})

Nutrient	NRC (1994)	ISA (1996)	Diet 1	Diet 2	Diet 3	Diet 4
Crude Protein	150	195	195	195	195	195
ME (MJ kg^{-1})	12.1	11.8	11.4	12.0	12.3	12.6
Calcium	32.5	37	37	37	37	37
Av. Phosphorus	2.5	3.6	3.8	3.8	3.8	3.8
Av. Lysine	6.9	8.8	7.8	7.9	7.9	7.9
Met + Cys	5.8	7.2	6.4	6.4	6.4	6.4

2.2.5. Experimental design

The design was a 4 X 2 factorial with 6 replicates per treatment and 2 birds per replicate. The treatments were the four levels of ergot alkaloid (1) 24 $mgkg^{-1}$, (2) 12 $mgkg^{-1}$, (3) 6 $mgkg^{-1}$, and (4) 0 $mgkg^{-1}$ and the two levels of Mycosorb (with and without this binding agent) (Table 3a (i),(ii)).

Table 3a (i). Treatment factors

Factor 1	x	Factor 2
Ergot Alkaloid Level ($mgkg^{-1}$)	x	Binding Agent Level
1. 24		a. + 0.2 gkg^{-1} Mycosorb
2. 12		b. No Mycosorb
3. 6		
4. 0		

Table 3a (ii). Diet codes

DIET	SE	MYCOSORB
1a	24	+
1b	24	-
2a	12	+
2b	12	-
3a	6	+
3b	6	-
4a	0	+
4b	0	-

The eight treatments were randomly allocated to the 48 cages so that there were six replicates of each treatments (Tables 3b (i), (ii)).

Table 3b (i). Randomization of treatments

2b	1b	2b	1b	3b	4b	2a	4b	4b	4a	3a	4b	1b	4b	1b	1b	3a	3a
31	32	33	34	35	36	37	38	39	40	41	42	43	44	45	46	47	48
13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30
1a	4a	2b	4a	3a	3b	2a	3a	2a	1b	2b	1a	3b	4a	1a	4a	2b	4a

2a	2b	1a	4b	3b	3b	2a	3a	1a	3b	2a	1a
1	2	3	4	5	6	7	8	9	10	11	12

Table 3b (ii). Replicate locations (Pen number)

Treatment	**Replicate**					
	1	**2**	**3**	**4**	**5**	**6**
1a	3	9	12	13	24	27
1b	22	32	34	43	45	46
2a	1	7	11	19	21	37
2b	2	15	23	29	31	33
3a	8	17	20	41	47	48
3b	5	6	10	18	25	35
4a	14	16	26	28	30	40
4b	4	36	38	39	42	44

2.2.5.1. Parameters measured

The following measurements were taken.

- Egg production - Eggs were collected daily. The results were calculated as hen day egg production per week (total egg production/ number of birds per cage).
- Egg weight - Egg weight per egg per cage was measured in grams once a week (days 7, 14, 21, 28, 35, 42).
- Egg mass - Egg mass per bird per cage was calculated as (Production % x Mean egg weight).
- Feed intake - Feed intake per bird per cage was measured in grams weekly as the (amount feed given-residue)/ number of birds per cage.
- Feed Conversion Ratio (FCR) - FCR was calculated each week as feed intake/ egg mass.

2.2.5.2. Statistical analysis

The measurements were analysed using ANOVA (SAS Institute, Inc.1990) and the result of each treatment was expressed as mean ± standard errors of the mean. The significant differences between each mean were calculated using Tukeys test (Steel and Torrie, 1980).

2.3. Results

The effects of sorghum ergot alkaloid on the production of the ISA Brown laying hens are shown in tables 4,5,6,7 and 8. The results recorded during the six weeks trial were the performance of egg production, egg weight, egg mass, feed intake and FCR.

The total egg production for the six weeks was significantly less for the birds fed the 24 mgkg-1 DHES diet than for birds fed any of the other diets. There was no significant difference between the egg production of birds fed 12, 6 or 0 mgkg-1 DHES.

There was no significant effect of sorghum ergot alkaloid on egg production in any week except week 5, when the diet with 24 mgkg-1 DHES produced significantly ($P<0.05$) lower egg production than the diet with 6 mg/kg DHES. However, there was a trend for the egg production to be less each week for birds fed the highest ergot diets. There was also a significant decrease in egg production over the six weeks, with production in week 6 more than 12% lower than in week 1(Table 4).

The total egg production of birds fed Mycosorb over six weeks was significantly ($P<0.01$) greater than that of birds not fed Mycosorb in the diet. However, there was no significant difference between the egg production of hens fed diets with or without Mycosorb in any one week (Table 4).

There were some significant interactions between ergot alkaloid level and Mycosorb addition in week 3 – 6. For example, hens fed the diets containing 6 and 12 mg/kg DHES with Mycosorb had significantly ($P<0.05$) greater egg production than hens fed one of the diets contain 24 mg/kg DHES with or without Mycosorb in weeks 3 to 6 (Table 4).

Table 4. Egg production per bird fed various levels of ergot alkaloid for 6 weeks (%HD)

Treatment		Week						Mean
DHES	Myco Sorb	1	2	3	4	5	6	
24	+	85.71	78.57	83.36^{ab}	64.28^{b}	58.33^{b}	76.20^{ab}	74.40^{cd}
	-	82.14	77.38	69.05^{b}	70.23^{ab}	63.11 ^{ab}	63.10^{b}	70.83^{d}
12	+	88.10	89.28	90.48^{a}	86.91^{a}	80.95 ^{a}	83.35^{ab}	86.51^{a}
	-	83.33	76.19	76.21^{ab}	66.67 ^{ab}	63.11 ^{ab}	72.63^{ab}	73.02^{d}
6	+	91.69	90.47	90.50^{a}	82.15 ^{ab}	80.96^{a}	86.91^{a}	87.11^{a}
	-	86.90	84.52	78.56 ^{ab}	77.38 ^{ab}	75.00^{ab}	71.41^{ab}	78.96^{bc}
0	+	86.90	83.33	80.96^{ab}	64.30^{b}	76.18^{ab}	70.23^{ab}	76.98^{cd}
	-	91.66	83.34	86.91^{ab}	80.98 ^{ab}	77.40^{ab}	75.01^{ab}	82.55^{ab}
Mean Myco Sorb	+	88.10	85.41	86.32	74.41	74.10	79.17	81.25*
	-	86.00	80.35	77.68	73.81	69.65	70.53	76.33
Mean DHES	24	83.93	77.97	76.20	67.25	60.72^{y}	69.65	72.62^{y}
	12	85.71	82.73	83.35	76.79	72.03^{xy}	77.99	79.76^{x}
	6	89.28	87.50	84.53	79.76	77.98^{x}	79.16	83.03^{x}
	0	89.28	83.33	83.93	72.64	76.79^{xy}	72.62	79.76^{x}
Mean per week		87.05	82.88	82.00	74.11	71.87	74.85	78.79
	±SE	±4.59	±6.68	±6.53	±7.44	±8.23	±7.62	±6.84

*, a, b,c,d , x, y Within the same column, means with the same superscript or no superscript are not significantly different (P<0.05).

There was no significant effect of sorghum ergot alkaloid on egg weight in any week except week 4, when the diet with 24 mg/kg DHES produced significantly lower mean egg weight than the diet with 12 mg/kg DHES. However, there was a trend for the egg weight to be less in birds fed the higher ergot diets.

There was no significant effect of Mycosorb on egg weight in any one week or over the total six week production (Table 5).

There were some significant interactions between ergot alkaloid level and Mycosorb addition for egg weight in week 1 and 6. (Table 5).

Table 5. Average egg weight (g) from ergot alkaloid experiment during 6 weeks.

Treatment		Week						Mean
DHES	Myco sorb	1	2	3	4	5	6	
24	+	61.47[ab]	48.60	60.71	49.55	50.71	63.40[a]	55.74
	-	64.39[a]	53.46	53.20	52.50	65.36	64.01[a]	58.82
12	+	62.71[a]	63.02	63.61	65.48	63.71	65.46[a]	63.99
	-	60.36[b]	45.92	62.91	61.90	52.95	51.75[b]	55.96
6	+	58.39[b]	60.83	60.83	62.81	61.66	61.55[ab]	61.01
	-	64.44[a]	62.99	63.11	62.33	53.60	63.98[a]	61.74
0	+	63.93[a]	59.81	63.65	55.48	53.16	66.36[a]	60.39
	-	63.19[a]	69.41	63.77	61.30	63.55	64.56[a]	64.29
Mean Myco sorb	+	61.61	58.06	62.20	58.33	57.31	64.19	60.28
	-	63.09	57.94	60.74	59.50	58.86	61.07	60.20
Mean DHES	24	62.93	51.03	56.95	51.02[y]	58.04	63.70	57.27
	12	61.53	54.47	63.26	63.69[x]	58.33	58.60	59.98
	6	61.42	61.91	61.97	62.57[x]	57.63	62.76	61.37
	0	63.56	64.61	63.71	58.39[xy]	58.35	65.46	62.34
Mean per week		62.35	58.00	61.47	58.91	58.08	62.63	60.24
	±SE	±1.15	±10.38	±4.08	±6.59	±7.59	±3.85	±5.60

a, b, x, y Within the same column means with the same superscript or no superscript are not significantly different (P<0.05)

The average egg mass of birds fed the diets containing 24 $mgkg^{-1}$ DHES for six weeks was a highly significantly (P<0.01) less than that of birds fed the lower DHES diets.

There was a significant effect of sorghum ergot alkaloid on egg mass in week 3 and 4, when the diet with 24 mg/kg DHES produced significantly (P<0.05) lower egg mass than the diet without DHES and the diets with 6 and 12 $mgkg^{-1}$ DHES respectively.

There was a significant effect of Mycosorb on egg mass. Hens fed the diets with the Mycosorb supplement had significantly (P<0.01) greater egg mass than hens fed the diets without Mycosorb.

There were some significant interactions between ergot alkaloid level and Mycosorb addition on egg mass in week 3, 4 and 5 (Table 6).

Table 6. Egg mass (g) from ergot alkaloid experiment during 6 weeks

Treatment		Week						Mean
DHES	Myco Sorb	1	2	3	4	5	6	
24	+	52.58	41.88	50.66^{a}	34.51^{b}	30.96^{b}	48.41	43.16^{cd}
	-	52.52	44.74	35.81^{b}	37.61^{b}	41.13^{ab}	40.11	41.98^{d}
12	+	55.30	56.39	57.60^{a}	57.10^{a}	51.48^{a}	54.70	55.42^{a}
	-	50.29	38.45	48.15^{a}	41.61^{ab}	36.28^{ab}	39.11	42.31^{d}
6	+	53.64	55.00	54.96^{a}	51.53^{ab}	50.06^{ab}	53.50	53.11^{ab}
	-	55.96	53.45	49.63^{a}	48.21^{ab}	40.51^{ab}	45.98	48.95^{b}
0	+	55.53	50.09	52.45^{a}	36.55^{b}	42.85^{ab}	46.85	47.38^{bc}
	-	57.99	56.79	55.25^{a}	49.63^{ab}	49.61^{ab}	48.41	52.94^{ab}
Mean Myco sorb	+	54.26	50.84	53.91	44.92	43.83	50.86*	49.77*
	-	54.19	48.35	47.21	44.26	41.88	43.40	46.54
Mean DHES	24	52.55	43.10	43.24^{y}	36.06^{y}	36.05	44.26	42.54^{y}
	12	52.79	47.42	52.87^{xy}	49.35^{x}	43.88	46.90	48.86^{x}
	6	54.80	54.22	52.30^{xy}	49.87^{x}	45.29	49.74	51.03^{x}
	0	56.76	53.44	53.85^{x}	43.09^{xy}	46.23	47.63	50.16^{x}
Mean per week		54.22	49.59	50.56	44.59	42.85	47.13	48.15
	±SE	±2.87	±8.32	±5.20	±6.51	±7.48	±5.78	±6.02

a, b, c,d, x, y Within the same column, means with the same superscript are not significantly different ($P<0.05$)
* significantly greater ($P<0.01$).

There was no significant effect of sorghum ergot alkaloid on feed intake in any week for the total six weeks. However, there was a trend for the feed intake to be less in birds fed the highest ergot diets except in week 5.

There was no significant effect of Mycosorb in any week, and the significant interaction between ergot alkaloid level and Mycosorb in week 3 and over the total six weeks, did not show any gradation with ergot alkaloid concentration (Table 7).

Table 7. Average feed intake (g/bird) in ergot alkaloid experiment during 6 weeks

Treatment		Week						Mean
DHES	Myco Sorb	1	2	3	4	5	6	
24	+	86.91	85.71	94.15[ab]	89.41	106.25	97.65	93.34
	-	92.52	80.29	95.23[ab]	84.85	100.25	90.18	90.55
12	+	101.87	95.09	106.68[ab]	98.43	106.33	105.55	102.32
	-	84.83	84.59	85.76[b]	86.43	93.43	91.48	87.75
6	+	98.19	87.03	93.36[b]	86.05	97.35	98.60	93.43
	-	97.98	85.69	98.58[ab]	98.36	100.66	102.85	97.35
0	+	96.36	85.14	82.76[b]	82.68	95.70	96.96	89.93
	-	105.09	92.10	109.95[a]	98.50	98.35	105.66	101.60
Mean Myco sorb	+	95.83	88.24	94.23	81.68	101.40	99.69	93.51
	-	95.10	85.66	97.38	92.03	98.17	97.54	94.31
Mean DHES	24	89.72	83.00	94.69	87.13	103.25	93.91	91.95
	12	93.35	89.84	96.22	92.43	99.88	98.51	95.03
	6	98.08	86.36	95.95	92.20	99.00	100.72	95.38
	0	100.72	88.62	96.35	90.59	97.02	101.31	95.76
Mean per week		95.46	86.95	95.80	86.85	99.78	98.61	93.90
	±SE	±7.09	±5.97	±5.97	±6.11	±6.58	±6.04	±6.29

a, b, Within the same column, means with the same superscript or no superscript are not significantly different ($P<0.05$)

There was no significant effect of sorghum ergot alkaloid on feed conversion ratio in any week. There was no significant effect of Mycosorb in any week. There was no significant interaction between ergot alkaloid level and Mycosorb addition in any week (Table 8).

Table 8. Feed conversion ratio from ergot alkaloid experiment during 6 weeks.

Treatment		Week						Mean
DHES	Myco Sorb	1	2	3	4	5	6	
24	+	1.64	1.50	2.00	2.05	2.65	2.18	2.00
	-	1.76	1.30	1.86	1.61	2.63	2.55	1.95
12	+	1.85	1.69	1.85	1.76	2.08	1.95	1.86
	-	1.69	1.72	1.86	2.65	1.90	2.30	2.02
6	+	1.83	1.60	1.68	1.70	1.98	1.86	1.77
	-	1.75	1.62	2.03	2.05	1.85	2.40	1.95
0	+	1.79	1.83	1.76	1.70	1.70	2.26	1.84
	-	1.81	1.96	2.11	2.08	2.16	2.23	2.05
Mean Myco sorb	+	1.77	1.65	1.82	1.80	2.10	2.06	1.86
	-	1.75	1.65	1.96	2.09	2.13	2.37	1.99
Mean DHES	24	1.70	1.40	1.93	1.83	2.64	2.36	1.96
	12	1.77	1.70	1.85	2.20	1.99	2.12	1.93
	6	1.79	1.61	1.85	1.87	1.91	2.13	1.86
	0	1.80	1.89	1.93	1.89	1.93	2.24	1.94
Mean per week		1.76	1.65	1.89	1.94	2.11	2.21	1.92
	±SE	±0.12	±0.34	±0.24	±0.39	±0.39	±0.44	±32

No significant differences

2.4. Discussion

Sorghum contaminated with ergot was the base grain used in this experiment. The analysed protein level of the contaminated sorghum was higher (147 gkg^{-1} CP) than the crude protein of the non contaminated sorghum (89 gkg^{-1} CP). These differences were balanced by the use of soybean meal so that all diets had equal CP levels. The nutrient levels used in this trial lay between those recommended by NRC (1994) and ISA (1996)(Table 3).

Egg production was the main parameter measured in this experiment because egg production represents the main economic factor of laying hens in relation to their use of feed. High DHES concentration had an effect on egg production. Egg production from the diet containing 24 $mgkg^{-1}$ DHES was significantly less than that produced from the other diets. This result agrees with the findings of Bandyopadhyay *et al*., (1998) that egg production was adversely affected at higher levels of ergot alkaloids. The addition of Mycosorb® as a binding agent to the diets was beneficial, because the egg production of laying hens fed with sorghum ergot plus Mycosorb was 6.4% greater than for non-supplemented hens overall (Table 4).

The decrease in mean egg production over the six weeks of the trial was greater than expected for ISA Brown hens of this age (ISA, 1998). The decrease was 87% to 74.8% in this trial whereas the ISA manual (1998) indicates normal production decreases from 81% to 78.8% at age 60 to 66 weeks.

Egg weight was not significantly affected by the levels of DHES, but there was a trend of decreasing egg weight as ergot concentration in the feed increased.

As with egg production and egg weight, egg mass decreased as the levels of DHES in the diets increased. Egg mass was significantly increased by the addition of the Mycosorb to the feed.

Overall feed intake was less than expected for ISA Brown hens. Levels of DHES did not significantly affect feed intake but the feed intake of birds was least when fed the diets containing the highest level of DHES. This latter situation agreed with the finding of Rotter *et al*., (1985ab) that feed consumption was reduced with the presence of ergot in the diet. DHES concentration did not appear to have a great effect on feed conversion ratio. In treatments showing trends, a more definitive answer may be able to be obtained using a larger number of replicates and a longer period of feeding.

The practical application of these results is to recommend not feeding more than 12 $mgkg^{-1}$ DHES to laying hens. Present regulations permit 0.3% sorghum ergot (approximately 1 $mgkg^{-1}$ DHES). However, increasing the maximum limit to 1% sorghum ergot (approximately 5 $mgkg^{-1}$ DHES) would not significantly affect the production or efficiency of laying hens.

Most batches of sorghum do not contain high levels of ergot. Those with levels higher than 0.3% ergot are withdrawn from sale but the number of these is few at present (Blaney, personal communication). If the allowable level of contamination of sorghum with ergot is raised to 1% it is likely that most, if not all, batches of sorghum would pass inspection and be suitable to feed to laying hens without significantly increasing the risk of affecting production.

2.5. Conclusion

Feeding sorghum contaminated with high levels of ergot tends to decrease egg production and egg mass and the addition of Mycosorb prevents the effects of ergot alkaloid on egg production and egg mass.

This finding suggests that if sorghum is contaminated with ergot either layer feed should be supplemented with Mycosorb®, and/or the concentration of DHES in the diet should not be greater than 12 $mgkg^{-1}$.

Increasing the maximum allowable concentration of ergot in layer diets from 0.3 to 1% (from approximately 1 mg to 5 mg DHES/kg diet) would not significantly affect the production or efficiency of laying hens and would provide a safe outlet for ergot contaminated sorghum, provided the ergot alkaloid was not deposited in eggs.

3. The effect of sorghum ergot alkaloid on the dry matter digestibility of laying hens

3.1. Introduction

3.1.1. Alkaloids

The alkaloid content of *C. africana* varies between 0.02 – 0.98 % w/w. The alkaloid dihydroergosine (DHES) accounts for 88% of total alkaloids in sorghum. Diets with up to 50 % sclerotia of *C. africana* (15 mg of alkaloid per day) when tested on mice were harmless and did not induce clinical effects. Toxic effects of ergot alkaloids were noticed in chickens fed with diets containing 2.5 and 5.0% sclerotia in feed. The chickens exhibited respiratory difficulties and diarrhoea, and died. However, with diets containing 1.25 % sclerotia, no significant clinical effects were reported by Bandyopadhyay *et al.* (1998). Higher dietary ergot levels (0.4 to 9.0 %) resulted in a depression in growth and increased chick mortality. Laying hens were shown to be more tolerant to dietary ergot (9%) than chicks, but egg production was adversely affected at higher levels of ergot alkaloids.

Burferning (1973) indicated that loss of appetite and increased body temperature could be caused by ergot alkaloids affecting the appetite control centre and temperature control centre in the hypothalamus.

Alkaloids comprise a wide range of different substances, many with valuable pharmaceutical properties (ergotamine is used to treat migraine headaches), and a few of which are poisonous to livestock. Poultry have tolerated up to 1 % of ergot by weight in the complete diet. Queensland Stockfeed regulations limit ergot in stockfeed to 0.02 %, which covers all varieties of ergot (Blaney, 1998). Sorghum ergot produces a different range of alkaloids than the ergot of rye (*C. purpurea*) and their effects appear to be less severe. In 1997 the stockfeed regulations in Queensland were changed to increase the amount of sorghum ergot that could be fed to livestock to 0.3%.

The rye ergot alkaloids act like dopamine, serotonina and noradrenaline agonist and antagonist, producing a cascade of physiological effects including reduced peripheral blood circulation (Mantle, 1977). Rotter *et al.,* (1985a,b) also reported that the characteristic signs of increased rye ergotism in broilers are reduced feed consumption, depressed growth, incoordination, poor feathering and vasoconstriction resulting in elevated blood pressure, restricted blood flow and subsequent necrosis of toes, beak and skin.

3.1.2. Digestibility

Digestibility is important in determining overall nutritive value. The presence of undesirable factors like ergot alkaloids in sorghum may influence the dry matter digestibility. Pethick *et al*., (1999) stated that sorghum grain is well digested by poultry. The whole tract digestibility of dry matter by poultry is 85 % (Pethick *et al.*, 1999). There has been limited research in poultry especially in laying hens into the effect of sorghum ergot alkaloid on dry matter digestibility. Poor digestibility of dry matter by poultry will affect the nutrient intake, metabolizable energy and carbohydrate, protein and fat digestibility (Pierson *et al*., 1980).

As little as 0.06 % ergot in the ration of cattle could affect animal performance and physiological functions. Studies on calves fed ergot-infected sorghum show reduction in feed intake and reduced weight gain despite no significant decrease in apparent digestibility of dry matter (Dinusson *et al*, 1980).

3.1.3. Objectives

The first objective of this study was to determine the effect of sorghum ergot alkaloid (SEA) on the dry matter digestibility of laying hens. It is important in studying the metabolism of ergot alkaloid in poultry to know something about the general digestibility of the nutrients in the diets (i.e. how much of the diet is absorbed and how much is excreted) so that an estimate can be made of how much ergot alkaloid might be present in the body and able to be excreted into the egg. Secondly, it is important to know if ergot alkaloids decrease the digestive efficiency of poultry.

The second objective was to determine whether the binding agent Mycosorb® modified the effect of sorghum ergot alkaloids on the digestibility of dry matter.

3.2. Materials and methods

3.2.1. Birds

A total of 32 commercial ISA Brown laying hens, 66 weeks of age was used in this experiment at the University of Queensland, Gatton Campus.

3.2.2. Housing

The birds were housed in a semi-controlled environment. Dry and wet bulb mercury thermometers were used to measure maximum temperature and humidity. Maximum temperature was 20^{o}C and relative humidity was 74%. A small fan operated continuously to ventilate the room, which had open louvres on the southern side. Fluorescent light was controlled by a time clock to provide 16 hours light and 8 hours of dark.

3.2.3. Experimental feed

Four basal layer diets were formulated using sorghum, fishmeal, vegetable oil, limestone, vitamin & mineral mix, DL-Methionine and L-Lysine (UFFF, 1986)(Table1). Ergot contaminated sorghum analysed to contain 32 mgkg^{-1} dihydroergosine (DHES) was used as 760 gkg^{-1} of the diet. This means that basal Diet 1 was estimated to contain 24 mgkg^{-1} DHES. Ergot contaminated sorghum was diluted 50% twice with normal uncontaminated sorghum to produce Diet 2 to contain 12 mgkg $^{-1}$ DHES, and diet 3 to contain 6 mgkg^{-1} DHES. Diet 4 contained uncontaminated sorghum only. The ingredient composition and nutrient analysis of the experimental diets are shown in Tables 9 and 10. The DHES content of Diet 1 was analysed to be 19 mgkg^{-1} diet. Half of these four basal diets were mixed with Mycosorb® to study the effect that this binding agent had on DM digestibility.

Table 9. The ingredient composition of the experimental diets (glkg^{-1})

Ingredient	Diet 1	Diet 2	Diet 3	Diet 4
Sorghum (ergot) (14.7% CP)	754.6	354.5	169.6	0
Sorghum (Non-ergot) (8.9% CP)	0	354.5	509.0	655.8
Fish meal (65% CP)	130	121.2	116.0	112.1
Soybean Meal (59% CP)	0	60	100	130
Vegetable Oil	20	18.6	17.8	17.2
Limestone	90	83.9	80.3	77.6
Vit & Min Premix*	2.0	2.0	2.0	2.0
Choline	2.0	2.0	2.0	2.0
DL-Methionine	2.0	2.0	2.0	2.0
L-Lysine	1.0	1.0	1.0	1.0
Yolk pigment	2.0	2.0	2.0	2.0

*The Vitamin and Mineral Premixes added the following (in mgkg^{-1}) to the diet: 39.2 IU D_3, 15 IU Vit A, 4.2 IU Vit E, 1.74 IU Vit K, 126.7 mg, 100.3 mg Zn, 415,9 mg Fe, 10.7 mg Cu, 0.14 mg S, 018 mg Se, 0.11 mg Co, 4.1 mg thiamine, 7.7 mg riboflavin, 50.4 mg niacin, 3.5 mg pyridoxine, 8.1 mg panthothenate, 0.19 mg folic acid, 11.31 IU choline.

Table 10. The ration analysis (calculated nutrient content of basal diets, gkg^{-1})

Nutrient	NRC (1994)	ISA (1996)	Diet 1	Diet 2	Diet 3	Diet 4
Crude Protein	150	195	195	195	195	195
ME (MJ kg^{-1})	12.1	11.8	11.4	12.0	12.3	12.6
Calcium	32.5	37	37	37	37	37
Av. Phosphorus	2.5	3.6	3.8	3.8	3.8	3.8
Av. Lysine	6.9	8.8	7.8	7.9	7.9	7.9
Met + Cys	5.8	7.2	6.4	6.4	6.4	6.4

3.2.4. Faeces collection

Faeces collection trays were attached under the selected cages for the 24 hours collected period. The total amount of faeces produced per cage over the 24 hours was weighed and the faeces samples were stored in a freezer until analysed for dry matter.

3.2.5. Dry matter measurement

Two replicates from each diet and faeces were taken to determine moisture and dry matter content. Sixteen cages of laying hens were used to assess faeces dry matter and sixteen samples of feed were also used to assess dry matter. Dry matter (DM) and moisture content (MC) in feed and faeces were determined by drying at 100^{O}C for 24h in a drying oven. Dry matter and moisture content of the faeces and feed samples were calculated as .

$$\text{DM content} = \frac{\text{Dry weight}}{\text{Wet weight}} \times 100$$

$$\text{Moisture content} = 100 - \text{DM content}$$

3.2.6. Digestibility measurement

The dry matter of the feed and faeces were analysed and the digestibility of dry matter (DMD) calculated as:

$$\text{DM Digestibility \%} = \left(\frac{\text{DM feed intake - DM faeces output}}{\text{DM feed intake}} \right) \text{X } 100$$

3.2.7. Experimental design

The design was a 4 X 2 factorial with 2 replicates per treatment and 2 birds per replicate. The treatments were four levels of ergot alkaloid (1) 24 $mgkg^{-1}$, (2) 12 $mgkg^{-1}$, (3) 6 $mgkg^{-1}$, (4) 0 $mgkg^{-1}$, and two levels of Mycosorb® (with and without this binding agent). Differences between individual treatment means were assessed using Tukey's test (Steel and Torrie, 1980).

3.3. Results

The dry matter contents of the diets fed and faeces collected and the calculated digestibilities of dry matter are given in Table 11. There was no significant effect of the level of sorghum ergot alkaloid (SEA) or Mycosorb® on the dry matter digestibility in laying hens (Table 12). The average digestibility of dry matter was 76. 64%. There was a trend of decreasing digestibility with increasing levels of ergot alkaloid in the diets, the diet without ergot alkaloid having a dry matter digestibility of 78.3% and the diet with 24 mg/kg ergot alkaloid having a dry matter digestibility of 74.3% (Table 13).

Table 11. Dry matter digestibility of sorghum based diets containing graded levels of DHES

DHES Mg/kg	Myco sorb	Diet	Feed intake (g/b)	DM % feed	DM intake (g/b)	Faeces output (g/b)	DM faeces (%)	DM output (g/b)	DM digt. (%)	Mean	Mean
		1a	123.67	88.94	109.99	120.475	24.25	29.22	73.43	72.64	
	+	1a	122.295	88.94	108.77	126.245	24.25	30.61	71.86		
24		1b	150.215	89.61	134.61	125.995	24.83	31.28	76.76	76.07	74.35
	-	1b	146.94	89.61	131.67	130.49	24.83	32.40	75.39		
		2a	95.42	89.17	85.09	91.71	21.56	19.77	76.32	79.41	
	+	2a	97.955	89.17	87.35	72.795	21.56	15.69	82.04		
12		2b	109.605	88.74	97.26	106.26	22.58	23.99	75.33	73.93	76.67
	-	2b	104.535	88.74	92.76	112.835	22.58	25.48	72.53		
		3a	145.98	89.68	130.91	132.87	23.61	31.37	76.04	74.18	
	+	3a	115.30	89.68	103.40	121.235	23.61	28.62	72.32		
6		3b	126.945	89.10	113.11	106.23	20.52	21.80	80.73	80.38	77.28
	-	3b	147.225	89.10	131.18	127.65	20.52	26.19	80.04		
		4a	96.775	89.28	86.40	75.405	21.68	16.35	81.08	77.48	
	+	4a	128.005	89.28	114.28	137.685	21.68	29.85	73.88		
0		4b	118.2	89.28	105.53	103.245	22.35	23.08	78.13	79.1	78.29
	-	4b	127.61	89.28	113.93	101.625	22.35	22.71	80.07		

No significant differences (P>0.05)

Table 12. Analysis of variance

Source	df	SS	MS	F Value	Pr > F
Total	7	113.81229375	16.25889911	2.27	0.1373
Alkaloid	3	33.35846875	11.11948958	1.55	0.2754
Mycosorb	1	9.01500625	9.01500625	1.26	0.2949
Basal*Mycosorb	3	71.43881875	23.81293958	3.32	0.0777
Error	8	57.40915000	7.17614375		
Total	15	171.22144375			

Table 13. Dry matter digestibility of layers fed different levels of DHES and Mycosorb

DHES (mg/kg^{-1})	+ Mycosorb	- Mycosorb	Mean alkaloid
24	72.6	76.1	74.3
12	79.4	73.9	76.7
6	74.2	80.4	77.2
0	77.5	79.1	78.3
Mean	75.9	77.4	

No significant differences (P>0.05)

3.4. Discussion

The most obvious finding was that dry matter digestibility in laying hens was not significantly affected by ergot alkaloid levels. However there was a trend of decreasing digestibility with increasing levels of ergot alkaloid in the diet. Mycosorb® binding agent did not affect digestibility.

The diet containing 24 $mgkg^{-1}$ ergot alkaloid with Mycosorb had the lowest dry matter digestibility of 72.6% and the diet containing 6 $mgkg^{-1}$ ergot alkaloid without Mycosorb had the highest dry matter digestibility of 80.4%.

The reason for the trend of decreasing digestibility with increasing DHES concentration in the diet may be due to the vasoconstriction effects of dihydroergosine decreasing the blood flow in the mesenteric blood vessels and hence decreasing the rate at which the absorbed products of digestion are conveyed away from the mucosal cells. This reasoning is supported by the findings by Mantle (1977) and Rotter (1985ab) that ergot has physiological effects on poultry, including vasoconstriction.

This trend was only evident using extremely high levels of ergot alkaloid (analysed at 19 $mgkg^{-1}$ DHES) that would be unlikely to be encountered under practical conditions. It would therefore be safe to recommend a somewhat higher allowable level of sorghum ergot in feedstuffs than allowed at present. The present recommendation of 0.3% ergot could be increased to 1% ergot (i.e from 1 $mgkg^{-1}$ DHES to 5 $mgkg^{-1}$ DHES diet) without significantly affecting the digestibility of the feed by laying hens.

3.5. Conclusion

Feeding ISA Brown laying hens with sorghum contaminated with ergot in diets containing up to 19 mg/kg DHES with or without Mycosorb® did not significantly affect the dry matter digestibility of laying hens. However, there was a trend of decreasing digestibility of dry matter with increasing levels of DHES in the diet. This may have been due to DHES causing vasoconstriction thus decreasing the blood flow in the mesenteric blood vessel and hence decreasing the absorption of digested nutrients from the gut. However increasing the maximum allowable limit of sorghum ergot in feed from 0.3% to 1% (equivalent to increasing DHES from 1 to 5 $mgkg^{-1}$ feed) would not significantly affect the digestibility of the feed by laying hens.

4. An investigation into whether feeding sorghum ergot (*Claviceps africana)* to hens produces alkaloid residues in eggs.

4.1. Introduction

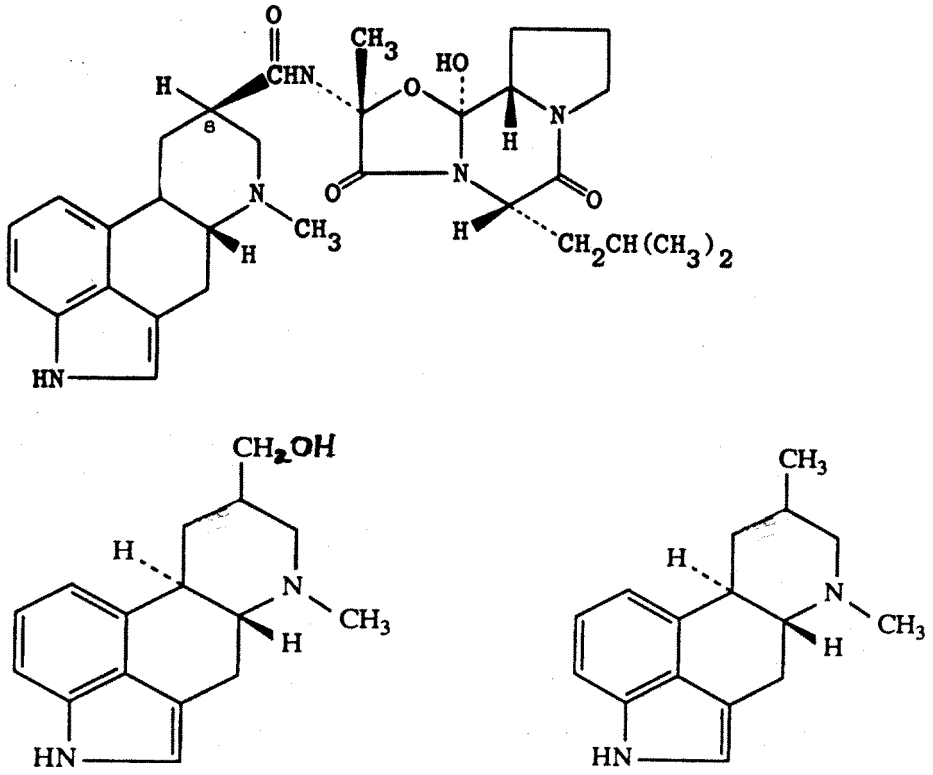
Sorghum ergot (*Claviceps africana)* is widespread in Africa and Asia and has recently been introduced into Australia (Ryley *et al*. 1996). The fungus infects sorghum at flowering and the infected florets eventually host the fungus as it develops hard sclerotia (ergots) that contain toxic alkaloids. The major alkaloids produced by sorghum ergot are dihydroergosine (DHES) which usually represents >80% of the total, festuclavine and dihydroelymoclavine. It should also be noted that the DHES molecule consists of an ergoline nucleus attached to a tripeptide moeity – the cyclol structure. Festuclavine and dihydroelymoclavine both lack the tripeptide side-chain, and are the biosynthetic precursors of DHES (Barrow *et al*. 1974). These three alkaloids are all saturated at the 9,10 position of the molecules, in which respect they differ from the various ergo-peptides produced by rye ergot (*C. purpurea*) such as ergotamine. Figure 1 shows the chemical structures of these compounds.

In general, the ergot alkaloids have toxicological properties relating to their pharmacological activity, which includes effects on the central nervous system, action on smooth muscle, and adrenaline, serotonin and dopamine antagonism. The dihydro-alkaloids are considered much less active in regard to vasoconstriction and endothelial damage than the parent alkaloids (Goodman and Gillman 1970). However, recent studies have shown that the sorghum ergot alkaloids (mainly DHES) have similar actions to rye ergot alkaloids in regard to inhibition of milk production in sows and cows, and producing hyperthermia and reduced growth in lot-fed cattle (Blaney *et al*. 2001). As with rye ergot alkaloids, chickens are more tolerant than other livestock species to sorghum ergot, but high concentrations of rye ergot (5-10%) in diets can produce gangrene of the comb. Effective use of infected sorghum might be achieved by feeding it to chickens or laying hens, but any residues occurring in eggs as a result might close this avenue.

There are no reports in the literature of the occurrence of residues in eggs as a result of feeding rye ergot. Whittemore *et al* (1976) investigated the consequences of feeding 4% rye ergot to growing pigs. Their balance experiments showed that 90% of ingested alkaloids (from 120mg/kg in diets, including 30% ergosine and 20% ergotamine) disappeared from the intestine, but that none could be detected in tissues or urine. They suggested that very efficient absorption of alkaloid was accompanied by equally efficient decomposition. Subcutaneous injection of DHES in mice resulted in 30% being recovered later in the faeces, presumably excreted in the bile (Mantle 1968).

This experiment was conducted to determine whether residues could be found in eggs from birds fed high levels of sorghum ergot (up to about 6%).

Figure 1. Structures of the sorghum ergot alkaloids



4.2. Materials and methods

4.2.1. Diet formulation

Sorghum grain infected with ergot was sourced from a farm in the Monto region of Queensland where cases of poisoning of pigs had been observed (Blaney *et al*. 2000). Ergot concentrations were estimated by visual separation and weighing of several sub-samples. The sorghum was hammer-milled and mixed extensively to ensure homogeneity. After hammer milling, subsamples were taken and assayed by high performance liquid chromatography using the method described in Blaney *et al*. (2003). The ergot content was estimated at 4-6%, and it contained 38 mg alkaloid/kg, including 32 mg dihydroergosine/kg, 5 mg dihydroelymoclavine/kg, and 1 mg festuclavine/kg.

Diets were formulated to include 0, 19, 38 or 75% of naturally ergot-contaminated sorghum and then steam-press pelleted. The diets were assayed and estimated to contain 0, 5, 9 or 19 mg DHES/kg. Part of this difference between actual and expected result might be ascribed to absorption of moisture after formulation.

4.2.2. Hens and husbandry

Isa Brown laying hens 60 weeks old were housed in double layered cages (two birds/cage) in a randomised layout and randomly allocated to diets. Water was available ad libitum. Temperature was controlled to 22°C.

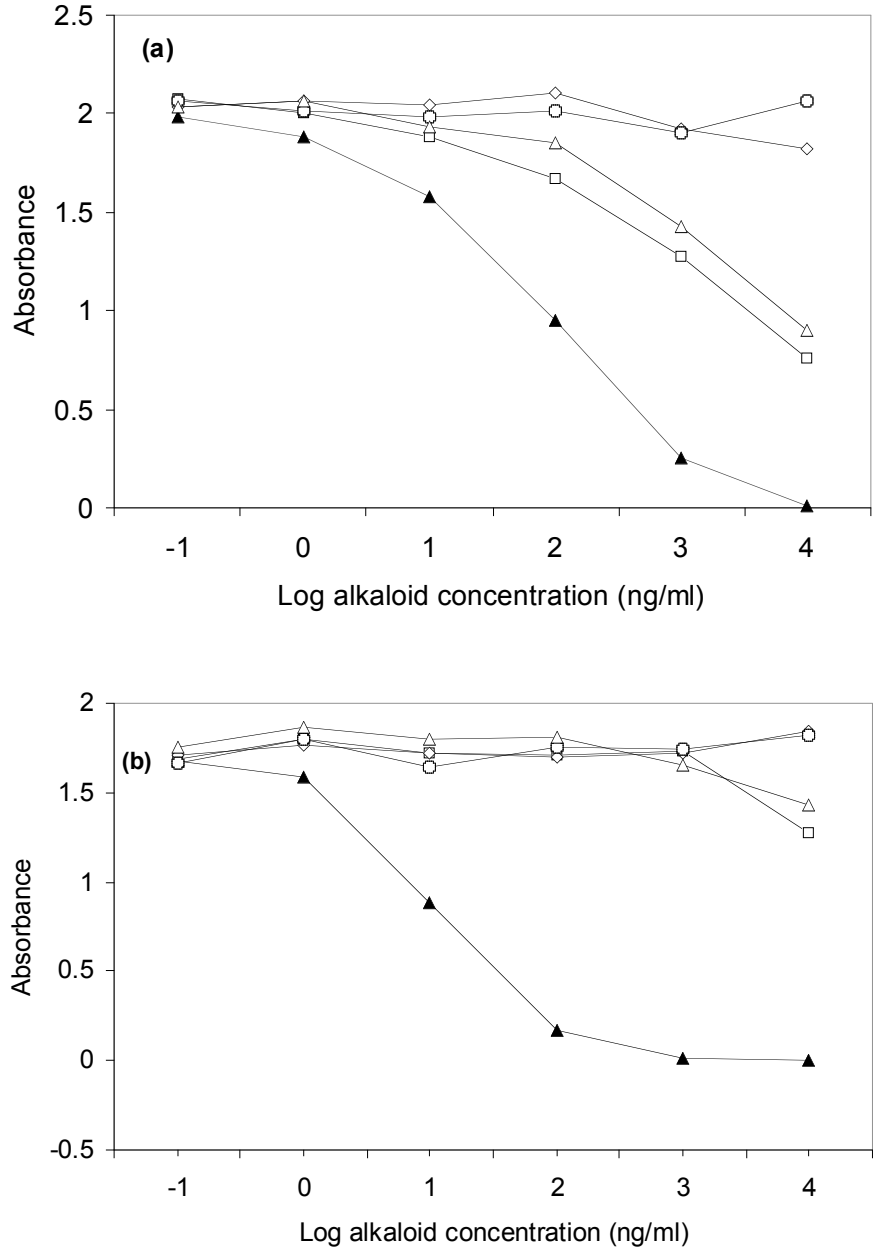
Eggs were collected daily, weighed and measured.

Excreta were collected over one 24 hour period from 16 cages, and digestibility of diets determined using standard techniques. The excreta (bulked within diets) were assayed for alkaloids using both ELISA and HPLC methods described below.

4.2.3. ELISA

Individual eggs (80 from hens fed 19 mg DHES/kg and 80 from control hens) were blended for 30 sec in a stainless steel Sorvall blender and stored at 4oC in plastic tubes. They were then diluted 1:1 with PBS and pipetted directly into wells, using a competitive ELISA based on a DHES-specific mouse monoclonal antibody and rabbit polyclonal antibodies raised against DHES conjugated to bovine serum albumin. These ELISAs have been fully validated over a wide concentration range by excellent correlation with a high performance liquid chromatography method (Molloy *et al*. 2003). The specificity is shown in Figure 2.

Figure 2. Specificity of ELISAs using (a) polyclonal rabbit antibody and (b) mouse monoclonal antibody, as shown by inhibition of antibodies at varying concentrations of dihydroergosine (▲), dihydroelymoclavine (◊), festuclavine (○), dihydroergotamine (□) and ergotamine (Δ).



The hapten antigen was produced by conjugation to BSA via the nitrogen atom on the ergoline nucleus. Subsequently, the resultant antibodies did not detect the clavine alkaloids festuclavine and dihydroelymoclavine, and also the 9, 10 dihydroergotamine reacted better than the 9,10 unsaturated ergotamine. From this it can be inferred from the specificity and the structures, that the resultant antibodies are reacting across the portion of the molecule containing the 9, 10 dihydro group, and the cyclol moeity.

The ELISAs were validated for whole egg by spiking a portion of 10 eggs (after blending) with the equivalent of 0.01mg DHES/kg (in aqueous solution) and comparing the ELISA results with a second portion of those (unspiked) eggs. These were assayed after 1, 3 and 7 days storage (at 4-10^{o}C).

4.2.4. High-performance liquid chromatography

Eggs (40 from hens fed 19 mg DHES/kg and 10 from control hens) were blended as above, and a 20 ml portion taken and mixed with 20 ml of phosphate buffer (pH6). This mixture was placed in an ultrasonic bath for 3 minutes, centrifuged for 30 min and frozen to facilitate separation of the protein plug. Next 10 ml of the supernatant was transferred to a Bond Elut C18 Solid Phase Extraction column (Varian^{T}) with 10 ml reservoir, that had been previously conditioned with 10 ml each of methanol and water. The column was washed with 10 ml of methanol: water (5:95). Alkaloids were then recovered with 5 ml methanol. This was reduced to 1 ml and filtered prior to HPLC separation. Recoveries were performed by spiking the egg with standard DHES at the 0.1 mg/kg concentration prior to extraction.

The HPLC system used was a Novapac (^{TM}Waters) C18 column 150 x 3.9 mm, used isocratically at 40^{o}C. The mobile phase was acetonitrile: methanol: 0.1% ammonium acetate (31: 20: 50). Detection was by fluorescence (Blaney *et al*. 2003).

4.3. Results and Discussion

4.3.1. Recoveries: ELISA

On all occasions, the eggs spiked with 0.01mg DHES/kg were very easily detected. Compared to standard DHES, the response ('recovery') was >100% of the standard, indicating that there was no significant interference with the assay such as binding of alkaloid to the egg components, and also some actual enhancement of response to DHES due to the egg matrix. However, there was a degree of variation in the result between eggs that was completely unrelated to ergot alkaloid, as it occurred in batches of eggs from birds with no ergot exposure – this variation amounted to about 0.08 OD unit, which was equivalent to a non-specific interference blank of a maximum of 0.005 mg/kg. This limited the detection limit to the same level of 0.005 mg/kg.

4.3.2. Recoveries: HPLC

The recoveries of DHES from eggs spiked at 0.1 mg/kg were 60-84%. The detection limit was 0.02 mg/kg.

4.3.3. Residues in eggs from treated hens

Over 80 eggs from a control group were compared with 80 eggs from birds fed 19 mg DHES/kg. No trace of alkaloid was detected in any of the samples that exceeded the non-specific interference level of 0.005 mg/kg. In order to allow for this non-specific variation between eggs, and to push the detection limit lower, the average optical density of readings for the 80 control eggs was compared with that for the 80 ergot-treated eggs. This indicated a very slight difference, and even if that difference represented alkaloid, then the ergot-treated eggs contained <0.002 mg/kg on average. Similarly, DHES was not detected in any of the 40 eggs from ergot-fed hens that were assayed by HPLC. In addition, there were no other peaks present in the chromatogram suggestive of alkaloids resulting from breakdown of DHES.

4.3.4. Absorption of alkaloid

Alkaloids were detected in the excreta at concentrations ranging from 1-10 mg DHES/kg on a wet matter basis. When compared with intakes, the recovery of DHES in the excreta ranged from 18- 39% (average 29%). In comparison, the dry matter digestibility of the diets averaged 76%.

4.3.5. Effect of ergot alkaloid on production

There was a small but significant decline in egg production in the birds fed the highest alkaloid concentration, which is dealt with in detail elsewhere. Birds fed 5 and 10 mg DHES/kg did not produce differently from controls.

4.4. Conclusions

It appears that the hens either absorbed or degraded 71% of ingested alkaloid, but that none of this appeared in the eggs. This is a similar result to that obtained by Whittemore *et al* (1976) with pigs. It might be that the cyclopeptide part of the ergopeptide molecule is particularly vulnerable to the digestive enzymes such as pepsin. If so, this might partly explain the greater sensitivity of ruminants to ergot alkaloids, since absorption could take place from the rumen, without exposure to high concentrations of proteolytic enzymes as with monogastrics. The most likely point of breakdown of the molecule would be to open and/or to remove the cyclopeptide group, and if so, one might expect to see a clavine product of similar structure to festuclavine or dihydroelymoclavine, which might also retain some biological activity. Such a product would probably not be detected by the ELISA, but should be extracted and appear in the HPLC chromatogram if they were present in eggs. No new peaks appeared in the chromatograms from ergot-treated eggs. Given that the high concentrations of sorghum ergot alkaloids used in this trial are highly unlikely to be used in practice, there appears no justification for further concerns over egg residues from feeding moderate levels of ergot. The

regulatory limit for ergot in feed for laying hens might be raised from 0.3% to at least 1% (from about 1 mg DHES/kg to about 5 mg DHES/kg) without significantly increased risk.

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