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Effect of avilamycin, tylosin and ionophore anticoccidials on *Clostridium perfringens* enterotoxaemia in chickens

Einfluß von Avilamycin, Tylosin und ionophoren Kokzidiostatika auf die *Clostridium perfringens*-Enterotoxämie des Huhnes

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Summary: In order to study the prophylactic and metaphylactic effect of antomicrobial growth promoters and ionophorous anticoccidials on the incidence of *Cl. perfringens* enterotoxaemia in chickens, experimental attempts were performed with 675 chickens in 27 trials. The birds were intraduodenally infected with *Cl. perfringens type A* (ATCC 3624). The following antimicrobial growth promoters and ionophore anticoccidials were used either on their own or in combination: avilamycin, narasin, monensin and tylosin.
While infected and non-medicated trials showed an average incubation period of 1 week, clinical symptoms occurred 2–4 days later in infected and medicated birds. Avilamycin medicated birds had the longest incubation period. In the infected and non-medicated trials, a mortality rate of 16 %–36 % was noted within 3 weeks *post infection.* The avilamycin trials showed a mortality rate of 0–8 % (0–2 birds died) and the narasin and monensin a mortality rate of 0–8 %, respectively. In the combination groups (monensin + avilamycin or narasin + avilamycin), the mortality rate ranged from 0 to 4 %. Tylosin showed a very good metaphylactic/therapeutic effect against *Cl. perfringens* enterotoxaemia.
Following infection, medicated birds showed a significantly better bodyweight gain than the chickens, whose feeds had not been supplemented. From epidemiological point of view, the systematic prevention of coccidiosis is a key in the control of *Cl. perfringens* enterotoxaemia in chickens.

Key words: Chicken, *Clostridium perfringens*-Enterotoxaemia, avilamycin, monesin, narasin, tylosin

Zusammenfassung: Um den prophylaktischen bzw. metaphylaktischen Einfluß von Leistungsförderern und ionophoren Kokzidiostatika auf das Auftreten der *Cl. perfringens*-Enterotoxämie des Huhnes zu studieren, wurden Untersuchungen in insgesamt 27 Versuchsgruppen mit 675 Broilerküken durchgeführt. Die Tiere wurden intraduodenal experimentell mit *Cl. perfringens Typ A* (ATCC 3624) infiziert. Als Leistungsförderer bzw. ionophore Kokzidiostatika kamen Avilamycin, Narasin, Monensin und Tylosin allein oder in Kombination zum Einsatz.
Während infizierte und nicht-medizierte Tiere durchschnittlich eine Inkubationszeit von ca. 1 Woche zeigten, waren die klinischen Symptome bei den medizierten Tieren 2–4 Tage später zu beobachten. Avilamycin-medizierte Tiere zeigten die längste Inkubationszeit. Bei infizierten und nicht-medizierten Tiergruppen war eine Mortalitätsrate von 16 %–36 % zu errechnen. Sie fiel in den medizierten Gruppen geringer aus. So war in der Avilamycin-Gruppe eine Mortalität von 0–8 % (0–2 Tiere) zu beobachten und die Narasin- sowie die Monensin-Gruppe wies jeweils eine Mortalität von 8 % auf. In den Kombinationen Monensin/Avilamycin bzw. Narasin/Avilamycin lag die Mortalität zwischen 0 und 4 %. Tylosin zeigt einen sehr guten metaphylaktischen bzw. therapeutischen Effekt auf die *Cl. perfringens*-Enterotoxämie. Bei den medizierten Tieren war unter dem Infektionsdruck eine bessere Tageszunahme zu verzeichnen als bei den infizierten Tiergruppen, die keine Futterzusatzstoffe erhielten. Epidemiologisch ist der konsequenten Kokzidienprophylaxe zur Bekämpfung der *Cl. perfringens*-Enterotoxämie die größte Aufmerksamkeit zu widmen.

Schlüsselwörter: Masthähnchen, *Clostridium perfringens-Enterotoxämie, Avilamycin, Monensin, Narasin, Tylosin*

Introduction

Clostridium perfringens enterotoxaemia of chickens was first described in 1961 and its occurrence has since been reported on numerous occasions worldwide. With a mortality rate from 1–12 %, at times even exceeding 25 %, the disease is almost always accompanied by coccidial infections, especially of the genus *Eimeria* (Parish, 1961; Bains, 1968; Vittos, 1974; Kattich et al., 1965; Nairn and Bamford, 1967; Gardinier, 1967; Bernier and Filon, 1971; Helmboldt and Bryant, 1971; Bickford, 1971; Huchzermeyer, 1972; Long, 1973; Köhler et al., 1974; Bernier et al., 1974; Long et al., 1974; Balauca, 1976; Kwatra and Chaudhury, 1976; Maxey and Page, 1977; Al-Sheikhly and Al-Saieg, 1980; Morch, 1973; Köhler et al., 1977; Dysktra, 1978; Shane et al., 1985; Kageyama et al., 1987; Baba et al., 1988).

Since the mid-1980s the disease has ceased to play a role under practical conditions and *Cl. perfringens* enterotoxaemia of chickens has since then received very little attention in international research. This applies especially to the reason for the absence of clinical outbreaks in broiler flocks.

The present paper reports on experimental studies into the following aspects:

- the effect of avilamycin and ionophore anticoccidials on *Cl. perfringens* enterotoxaemia,
- morbidity and mortality rates and
- bodyweight development before and after a *Clostridium perfringens* infection.

Materials and methods

Experimental infections were performed in 3 sets of 9 experiments each, involving a total of 675 broiler chicks of both sexes. The experimental design is shown in Table 1.

The basal ration consisted of a drug-free complete feed for ducklings in meal form as described in Vissiennon (1991). This diet, supplemented with the various feed additives in the concentrations shown in Table 1, was fed to the chicks from one day-old to the end of the experiences.

Table 1
Experimental design

Trials and additive	Experimental birds (n)	Concentration	Infectious dose of *Cl. perfringens*
Negative group	25	none	not infected
Positive group	25	none	3.2 × 107 CFU
Avilamycin	25	10 ppm	"
Narasin	25	70 ppm	"
Monensin	25	100 ppm	"
Tylan*)	25	0.5 g/l	"
Avilamycin Narasin	25	10 ppm 70 ppm	"
Avilamycin Monensin	25	10 ppm 100 ppm	"
Narasin Tylan*)	25	70 ppm 0.5 g/l	"

*) As tylosin tartrate; administered via the drinking water after the first mortality.

In order to record the effects of the feed additives on body weight gain, especially just before and after the infection, the birds were individually weighed every other day. The scales used were models PT 2100 and PT 6 by Sartorius AG (Göttingen).

The infection was performed with the *Cl. perfringens* type A strain 3624 of the American Type Culture Collection (Rockville; MD). This strain (ATCC 3624) is characterised by a very good capacity for α-toxin formation, thermolability and a relatively good pH stability. It is considered the classic infectious strain (Weiss and Strong, 1967).

The bacteriological preparation of the samples and the infection were performed by the procedures described by Vissiennon (1991) and Vissiennon et al. (1994b). The birds were infected intraduodenally 5 times according to the method of Arnold and Vissiennon (1991). The age at infection ranged from 16 to 25 days post hatch.

Dead birds were subjected to gross pathological and histological examinations as described by Vissiennon (1991).

Faecal samples taken on arrival and samples of liver, intestine and spleen were tested microbiologically for the usual pathogenic organisms.

The results were biostatistically analysed as follows:

- Duncan's test with significance level 0,5,
- Kruskal-Wallis test for the analysis of variance,
- Kolmogorov-Smirnov Gooodness of Fit test to test the normal distribution of standard deviation.

Results

The faecal samples taken on arrival of the chicks and the organs of the dead broilers were all negative for salmonellae. Moreover, there was no evidence of any other pathogenic organisms in the dead broilers.

Morbidity, clinical symptoms

The non-infected birds (negative group) in all three experiments showed no signs of an infectious disease. In contrast, the infected birds who had received no feed additive showed marked clinical signs such as profuse, watery diarrhoea, lethargy to apathy, rough plumage and inappetence as early as one week *post infection* on average.

In the medicated birds the outbreak of clinical disease was recorded 2–4 days later and the symptoms were generally milder. There was little impairment of general health and symptoms such as apathy and inappetence were relatively rare.

Mortality

In the infected and non-medicated trials, a mortality rate of 16 %–36 % was noted within 3 weeks *post infection.* The avilamycin trials showed a mortality rate of 0–8 % (0–2 died birds) and the narasin and monensin a the mortality rate of 0–8 %, respectively. In the combination groups (monensin + avilamycin or narasin + avilamycin), the mortality rate ranged from 0 to 4 %. Tylosin showed a very good metaphylactic/therapeutic effect against *Cl. perfringens* enterotoxaemia (Fig. 1).

Some initial losses were recorded after 4 days, but most deaths occured two weeks *post infection.* The pathomorphological examinations revealed typical lesions of *Cl. perfringens* enterotoxaemia: prestatic hyperemia of the parenchymatous organs, watery contents of the small intestine mixed wih gas bubbles, cellular infiltration of the propria mucosae, cystic dilatation of the Lieberkühn glands. Necrosis or haemorrhage of the intestinal mucous membranes was not observed (Vissiennon et al., 1994b).

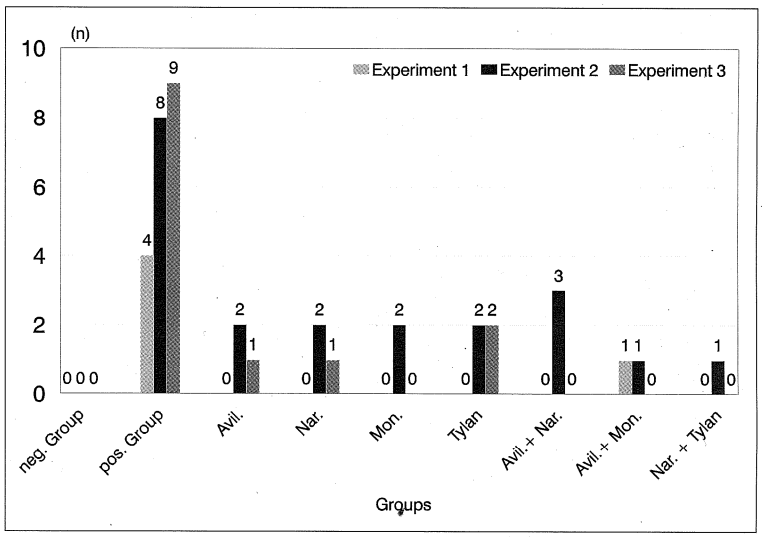

Fig. 1: Mortality rates (n) following experimental *Cl. perfringens* enterotoxaemia

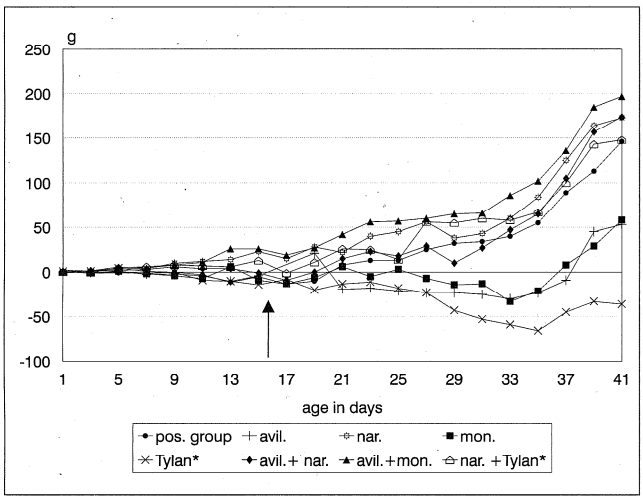

Fig. 2 Differences in average weights relative to the "negative group" (= x axis) in experiment 1 (The arrow shows the first day of infection)

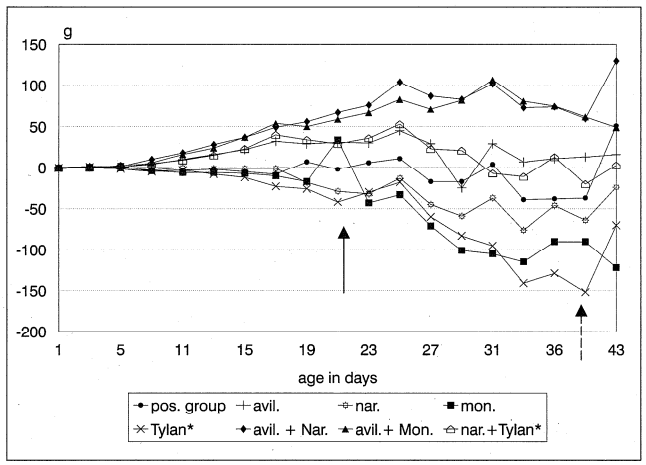

Fig. 3 Differences in average weights relative to the "negative group" (= x axis) in experiment 2. (The arrow shows the first day of infection, and the dotted arrow the first mortality and the begin of tylosin administration via drinking water)

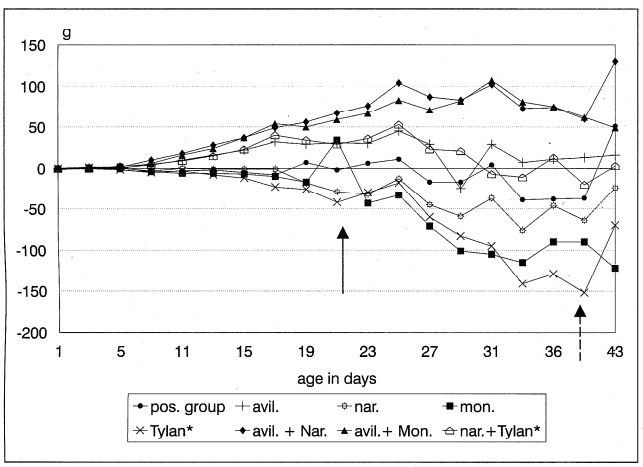

Fig. 4 Differences in average weights relative to the "negative group" (= x axis) in experiment 3. (The arrow shows the first day of infection, and the dotted arrow the first mortality and the begin of tylosin administration via drinking water)

Body weight development

The additives had a marked effect on bodyweight. Statistically significant differences were calculated from two weeks of age (Fig. 2–4). While the avilamycin-medicated groups (+) showed a slight weight depression post infection in the first experiment when compared with the negative control group, good weight gains were recorded in the two subsequent experiments (Fig. 3 and 4, arrow). The growth performance of the narasin- and monensin-medicated groups was curbed post infection, especially following the outbreak of clinical symptoms. The groups fed a combined supplement on the other hand (avilamycin + narasin or avilamycin + monensin) showed excellent weight gains post infection.

Following the tylosin medication, higher daily weight gains were observed, accompanied by a decrease of clinical symptoms, signs for a better feed conversion and for a increase of appetite (Fig. 3 and 4, dotted arrow).

Discussion

The *Clostridium perfringens* enterotoxaemia in the study reported here was induced experimentally. The post-mortem findings of the dead birds (severe hyperaemia of the parenchymatous organs, liquid to creamy gut contents permeated with gas bubbles) clearly indicate the consequences of *Cl. perfringens* enterotoxaemia. Previous transmission and scanning electron studies revealed scattered damage: mitochondrial damage, altered microvilli, plasma membrane bleb formation, disrupted terminal web, dilated endoplasmatic reticulum, and enterocyte detachment in the intestine (Vissiennon et al., 1993, 1994).

The toxins diffused and/or absorbed as a result of these ultramicrolesions in the gut enter the blood stream. Parenterally introduced α-toxin leads to transient hypotension by interfering with the Ca pump, bradycardia, reduced cardiac output (Stevens et al., 1988) and contraction of the smooth muscles (Sakurai et al., 1990). In vivo α-toxin provokes shock reactions with a lethal outcome (Stevens et al., 1988).

Ultrastructural findings include endothelial swelling both in the liver and in the kidney, thickening of the capillary basal membrane in the kidney and cell damage of varying degrees in both organs (Vissiennon et al., 1996). *Cl. perfringens* enterotoxaemia related mortalities in the absence of intestinal necrosis were reported as early as 1967 (Vittos, 1967).

The results of the present study demonstrate that experimentally induced *Cl. perfringens* enterotoxaemia can cause mortality rates of up to 36 %. Mortality rates of 100 % were observed in previous investigations (Vissiennon et al., 1994a).

These losses can be reduced considerably by using feed additives. With feed additives as employed in the present investigation, the mortality ranged from 0 to 8 %.

The groups with avilamycin in the feed performed well in terms of weight development, which is indicative of a good prophylactic and metaphylactic effect of the substance. Whereas infected and non medicated birds show wide fluctuation of the pH values of the intestine content, this has not been noted in avilamycin medicated cockerels as sign of eubiosis (Vissiennon, unpublished data). The efficacy of the substances in vivo can be assessed by the length of the incubation period, the severity of the clinical symptoms, the mortality rate and bodyweight development. Avilamycin prolongs the incubation period by 4 days on average compared with the non-medicated birds.

The prophylactic effect of avilamycin in terms of preventing mortality corresponds to that of the other drugs administrated in these experiments. However avilamycin stands out by its good "anti-*Cl. perfringens* enterotoxaemia activity" as evidenced by the weight development. The results of our study show that avilamycin medicated broilers withstand the infection.

The results for the narasin medicated group demonstrate good resistance to *Cl. perfringens* enterotoxaemia. The effect seems to be better than that of monensin. The good anticoccidial activity of the substance was investigated and assessed by several authors (Fairley et al., 1985; Jeffers et al., 1988a, b; Guneratne and Gard, 1991). According to studies by Wirenusz and Teeter (1991), Maxiban – a combination containing narasin – is unable to improve daily weight gains of male broiler chicks at temperatures of 24–35 °C, but does enhance feed conversion.

The polyether antibiotic monensin has good anti-*Cl. perfringens* enterotoxaemia activity as confirmed by the results of in vitro as well as in vivo studies. A relatively high concentration (5.5 µg/ml) is necessary to inhibit most of the *Cl. perfringens* strains tested by Kondo (1988). Yet monensin reduces the weight gain, compared to the non-medicated and non-infected birds. This is in agreement with the results of other authors (Keshavarz and McDougald, 1982; Parsons and Baker, 1982; Metzler et al., 1987; Vanderkop and MacNeil, 1980; Harms et al., 1989; Bartov, 1994).

Clinical outbreaks of *Cl. perfringens* enterotoxaemia (= necrotic enteritis) are almost always observed in association with (sub)clinical coccidiosis. Depending on the the coccidial species involved and the duration of exposure to the organisms, the pathomorphological picture of coccidiosis consists of a desquamative, fibrinous, haemorrhagic, diphtheroid to necrotising or necrotic enteritis of varying localisation (Wetzel, 1967; Long and Horton-Smith, 1968; Johannsen et al., 1986; Pohlenz, 1991; Vissiennon, 1991).

Coccidial infection is capable of inducing massive growth of anaerobic organisms in the intestine – especially *Cl. perfringens* – and of increasing the mortality rate (Visco and Burns, 1972a, b, c; Visco, 1975; Turk and Littlejohn, 1986; Vissiennon, 1991).

As a result of the increasing use of anticoccidials since the mid 1970s, outbreaks of clinically manifest coccidiosis with a lethal outcome have become very rare (Siegmann, 1983), and consequently reports of presumptive *Cl. perfringens*-induced necrotic enteritis.

Since 1985, Salisch et al. (1989) have not been able to demonstrate either coccidial oocysts in the litter or intestinal lesions in many broiler units. At the poultry abattoir of Osztyn (Poland) no coccidial infections have been diagnosed since 1989 after an original incidence of about 0.026 % in the years 1968–1988 (Radkowski et al., 1996). Köhler et al. (1977) observed an increase in necrotic enteritis as coccidia began to develop resistance to anticoccidials. In countries where antibiotic feed additives are banned (e.g. Sweden) high NE-related losses are still being recorded (Elwinger and Teglöf, 1991). However, the authors did not examine the dead birds for coccidial infection.

The uniform weight gains of the birds in the combination groups (avilamycin + narasin and avilamycin + monensin) is a major advantage in designing the medication regimen. It provides an alternative in the event of coccidia developing resistance to one of the two coccidiostats.

In view of this epidemiological development, it can be concluded that the pathomorphological term "necrotic enteritis" is closely linked to coccidial infections. Systematic coccidial prophylaxis is therefore the key element in the eradication of necrotic enteritis in poultry units and should be retained.

Conclusion

- In the absence of feed additives, high doses of *Cl. perfringens* type A organisms can trigger severe clinical disease in chicks with mortality rates of up to 36 %. The extent of the losses probably depends on the infectious dose, the age of the birds, the time of exposure to the pathogen, the protein and energy content of the diet and of the hygiene prevailing in the pens.
- By supplementing the diet with appropriate feed additives clinical outbreaks can be delayed by 2 days and the mortality rate can be reduced significantly. In our studies the mortality ranged from 0 to 8 %. Graduated differences were observed between the various active substances in morbidity rate, mortality rate and daily gains.
- With 10 ppm avilamycin in the feed clinical symptoms are observed later (by 4 d on average) than in the other groups. *Cl. perfringens* enterotoxaemia-related losses range from 0 to 2 chicks. Measured in terms of daily growth rates, avilamycin exhibits high resistance to *Cl. perfringens* enterotoxaemia *in vivo*.
- When using anticoccidials in practice, it is desirable to switch frequently between different substances in order to prevent the emergence of resistant coccidial strains. However, choosing the wrong drug can lead to lower daily gains. This does not happen with the combinations avilamycin + narasin or avilamycin + monensin.

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