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Effect of Antibiotic Growth Promoters and Anticoccidials on Growth of *Clostridium perfringens* in the Caeca and on Performance of Broiler Chickens

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Elwinger K, Berndtson E, Engström B, Fossum O, Waldenstedt L: Effect of antibiotic growth promoters and anticoccidials on growth of *Clostridium perfringens* in the caeca and on performance of broiler chickens. Acta vet. scand. 1998, 39, 433-441. – The effects of the growth promoters avoparcin and avilamycin and the ionophore anticoccidials maduramicin, narasin and monensin on the growth of *Clostridium perfringens* (Cp) in the ceaca and on performance of broiler chickens were tested in 2 experiments. The supplements were fed as single feed additives or in some combinations. No clinical signs or lesions caused by coccidia were observed in any of the studies. All supplements had an antibacterial effect on Cp and improved growth rate significantly. Carcass yield of birds fed growth promoters avilamycin or avoparcin was significantly higher compared with birds fed anticoccidials. These data indicate that, what concerns bird performance, during good hygienic conditions supplementation with antibiotic growth promoters may not be necessary when the diet is supplemented with an anticoccidial with antibacterial effects

feed additives; supplements; antibacterials; necrotic enteritis.

Introduction

Clostridium perfringens (Cp) is part of the normal bacterial flora in the gastrointestinal tract. Normally the number of Cp in the intestine is low. Under certain circumstances, however, Cp may multiply and cause enteric disease. Cp Type A has been shown to be a causal agent of necrotic enteritis (NE), an important disease of broilers throughout the world (*Ficken* 1991). NE occurs in the small intestine, but in earlier experiments (unpublished data) we have found that the development of Cp in the caeca is a good indicator of the number of Cp in the small intestine. Furthermore, sampling of caeca is easier and more practical than the small intestine.

Many antibiotic substances used as growth promoters, including avoparcin, virginiamycin (*Dutta & Devriese* 1980, *Kondo* 1988) and avilamycin (*Devriese et al* 1993), have been proven to have inhibitory effects on the growth of Cp. Also some anticoccidials, especially those of the ionophore type, have antibacterial activity (*Kondo* 1988, *Elwinger et al.* 1992). Due to the increasing problems caused by antibiotic resistant bacteria in human medicine and proven connection with the use of growth promoters in animal production (*Bates et al.* 1994, *Lange & Ek* 1995, *Howarth & Poulter* 1996, *Aarestrup et al.* 1996) it is urgent to decrease the use of feed antibiotics.

The purpose of the present trials was to study the effect of the growth promoters avoparcin and avilamycin and the ionophores maduramicin, monensin and narasin on the growth of Cp in caeca and the preventive effect against NE. The substances were used as single feed additives and in different combinations.

Materials and methods

Day old unsexed Ross broiler chickens were used. The chickens were randomly distributed in 11 m^2 pens, 145 in each, with wood shaving litter. Each treatment involved 6 pens (replicates). The composition and calculated nutrient content of the basal diet is shown in Table 1. All feeds were steam pelleted using a 3 mm die. During the first week the pellets were crushed to smaller particles. Three (Expt. 1) or 5 (Expt. 2) days before slaughter all birds were given feed without anticoccidial supplementation. The growth promoters were given during the whole experimental period in Expt. 1 and were withdrawn with the anticoccidials in Expt. 2. The concentration of the test substances in the diets was verified by chemical analyses.

The experimental periods were 46 (Expt. 1) and 45 (Expt. 2) days. Slaughter was carried out over the last 4 days, with equal number of pens per treatment each day. The withdrawal of anticoccidials from each pen was adjusted according to the day of slaughter. Prior to slaughter the birds were starved over night.

One chicken per pen was randomly selected and sacrificed by cervical dislocation at 2, 3, 4, 5 and 6 weeks of age for the control of Cp in caeca. Caecal samples of 2 birds per pen were taken from the processing line for control of Cp number. Processing yields were recorded by weighing the carcasses after evisceration and including the neck but not edible offal.

The dry matter content of the litter was determined in samples taken in the middle of each pen when the chickens were about 5 weeks of age. The foot pads were examined during processing to get an indication of the litter condition during rearing.

Table 1. Composition and calculated nutritional content of the basal diet fed to broiler chickens.

Ingredients	g/kg
Feed stoffs	
Barley	200.0
Wheat	419.4
Oats	100.0
Rapeseed meal 200	50.0
Soybean meal	120.0
Fish meal	50.0
Meat meal	20.0
Animal fat	15.0
Vit. and trace elements premix	10.0
Calcium carbonate	8.0
Sodium chloride	2.0
Dicalcium phosphate	3.0
DL-methionine	1 3
Lysine-HCL	1 3
Calculated nutritional content	
ME, MJ/kg	11.8
Protein	193
Lysine	11.0
Methionine	5 0
Met+cys	8.5
Fat	40
Linoleic acid	9
Calcium	9
Phosphorus	6.2
Potassium	7
Sodium	1 6
Chloride	2 6

Experiment 1

A total of 5220 birds were distributed in 36 pens. Avoparcin and avilamycin were added to the feed giving 15 and 10 mg/kg, respectively. The addition of anticoccidials was 5 mg maduramicin and 100 mg monensin per kg feed. The treatment of different groups is shown in Tables 2, 4 and 6.

Table 2. Main causes of mortality (no. of cases) among broiler chickens in experiment 1.

				Antibiot + anticoccidial			
		Antibiotics		Maduramicin		Monensin	
	Control	Avoparcin	Avilamicin	Avoparcin	Avilamicin	Avoparcin	Total
Yolk sac infection	6	4	16	7	10	11	54
Necrotic enteritis	13	0	0	0	0	0	13
Necrotic hepatitis	1	0	0	0	1	0	2
Ascites syndrome	9	17	16	6	10	15	73
Acute death syndrome	14	19	13	27	21	24	118
Skeletal deformations	1	2	3	2	1	3	12
Pericarditis	5	4	4	4	2	1	20
Miscellaneous	6	4	3	6	8	6	33
Negative section	7	6	1	1	5	1	21
Not autopsied	4	2	3	1	1	3	14
Total	66	58	59	54	59	64	360

Experiment 2

A total of 4350 birds were distributed in 30 pens. The concentrations of avoparcin and avilamycin in the feed were 15 and 10 mg/kg, respectively. The anticoccidials used were narasin (70 mg per kg feed) and monensin (100 mg per kg feed). The treatment of different groups is shown in Tables 3, 5 and 7.

Bacteriological examinations

Following sacrifice of chicks, caeca (including content) were collected aseptically, flamed, transferred to sterile plastic bags and macerated with sterile dilution fluid. Bacteriological examination of Cp was carried out as recommended by the *Nordic Committee on Food Analyses* (1985). Less than 10 (detection limit) colony forming units (cfu) per g caecum with content were calculated as 5 cfu per g. The Cp counts were transferred to $\log_{10}$ prior to statistical analyses.

Statistical analyses

Results were subjected to an analysis of variance using the GLM procedure in the Statistical Analyses System (*SAS Institute* 1989). At slaughter the data were adjusted to average age using Age as a block effect. Relative frequencies for e.g. mortality were angularly transformed (*Snedecor & Cochran* 1968) before statistical analyses. When significant difference(s) was found, the least significant difference (LSD) was calculated (*Snedecor & Cochran* 1968).

Results

Mortality causes are shown in Tables 2 and 3. The caecal carriage of Cp is shown in Tables 4 and 5, and the results of bird production performance are given in Table 7.

Average mortalities of 6.9% and 5.1% in Expt. 1 and 2, respectively were observed for the whole growing periods. There were no statistical significant differences between different treatment groups, and no signs of coccidiosis were observed. The main causes of mortality were acute death syndrome, ascites and yolk sac infections (Tables 2 and 3). The incidence of NE was on an average very low in both experiments, highest in Expt. 1 where all cases were found in the control pens (1.5%).

Table 3. Main causes of mortality (no. of cases) among broiler chickens in experiment 2.

		Antibiotics		Anticoccidials		
	Control	Avoparcin	Avilamycin	Monensin	Narasin	Total
Yolk sac infection	10	13	3	9	6	41
Necrotic enteritis	1	0	0	0	1	2
Ascites syndrome	5	5	4	6	8	28
Acute death syndrome	13	6	7	9	13	48
Skeletal deformations	18	7	8	10	11	54
Pericarditis	2	2	1	2	0	7
Constipation	2	2	2	2	2	10
Miscellaneous	3	4	7	4	2	20
Negative section	2	2	4	1	2	11
Not autopsied	8	6	9	6	7	36
Total	64	47	45	49	52	257

Table 4. Experiment 1. Analysis of caecal samples for *Clostridium perfringens* No. of positive birds out of no examined (no/no) and level of carriage as $\log_{10}$ cfu/g of caecal content

					Antibiot + anticoccidial			
			Antibiotics		Maduramicin		Mon-ensin	
Age days		Con-trol	Avo-parcin	Avila-micin	Avo-parcin	Avila-micin	Avo-parcin	To-tal
14	no/no	4/6	2/6	0/6	1/6	1/6	0/6	8/36
	log	2.8	1 3	0 7	1 5	0.9	0 7	1 3
21	no/no	5/6	0/6	2/6	1/6	1/6	0/6	9/36
	log	4 1	0.7	1.8	1.0	1 0	0 7	1 5
28	no/no	6/6	1/6	2/6	0/6	0/6	0/6	9/36
	log	6.2	1.8	1.0	0.7	0.7	0.7	1.8
35	no/no	6/6	0/6	2/6	2/6	2/6	0/6	12/36
	log	5 3	0 7	1 4	2.0	2.2	0.7	2.0
42	no/no	5/6	0/6	3/6	0/6	2/6	1/6	11/36
	log	4.5	0.7	2.2	0 7	1.0	0 8	1.7
43–46	no/no	12/12	7/12	8/12	11/12	11/12	6/12	55/72
	log	6.1	3.0	2 0	3 5	2 4	2.4	3 2

Counts of Cp from caecal content were significantly reduced by all test substances (Tables 4 and 5), and there was no significant difference between the drugs in this respect. Neither were any significant additional effects of the anticoccidials noticed when combined with the growth promoters. In Expt. 1, about 5 weeks of age, there were about 100% Cp positive samples from control birds, 17% from birds with avoparcin or avilamycin alone, and 22% from birds with avoparcin or avilamycin with anticoccidials, respectively. In Expt. 2 there were also 100% Cp positive samples from control birds, and an average of 17% from birds fed drug supplements.

The number of Cp positive birds and caecal

Table 5. Experiment 2· Analysis of caecal samples for *Clostridium perfringens*. No. of positive birds out of no. examined (no/no) and level of carriage $\log_{10}$ of cfu/g of caecal content.

			Antibiotics		Anticoccidials		
		Control	Avoparcin	Avilamycin	Monensin	Narasin	Total
14	no/no	4/6	1/6	6/6	6/6	5/6	22/30
	log	3.1	0.9	1.6	1.9	2.1	1.9
22	no/no	6/6	1/6	1/6	1/6	3/6	12/30
	log	6 4	0.9	1.0	0.9	2 5	2.3
29	no/no	1/6	0/6	0/6	0/6	1/6	2/30
	log	1 5	0.7	0.7	0.7	1.7	1.1
36	no/no	6/6	0/6	2/6	2/6	0/6	10/30
	log	7 1	0.7	2.5	2.8	0.7	2.7
42–45	no/no	9/12	11/12	10/12	12/12	12/12	54/60
	log	3 9	4.1	6.6	7.1	7.0	5.7

Table 6 Broiler chicken production performance in experiment 1.

					Antibiot + anticoccidial					
			Antibiotics		Maduramicin		Monensin	Statistical analyses[1]		
	Age, days	Control	Avoparcin	Avilamycin	Avoparcin	Avilamycin	Avoparcin	CV, %	p<	LSD
Mortality, %	37	6 1	5.5	5.2	5.3	5.9	5.4	19.0	.98	n s
	45	7.4	6.5	6.5	6.0	6.6	7.3	26.2	87	n.s.
Body weight, g	37	1857^{a}	1994^{b}	1976^{b}	1947^{b}	1963^{b}	1960^{b}	1.5	.001	59
	45	2303^{a}	2450^{b}	2405^{bc}	2390^{c}	2392^{c}	2390^{c}	1.4	.001	55
Carcass yield, %	45	72.4^{b}	73.3^{ab}	$73\ 4^{ab}$	72.8^{ab}	73.8^{a}	$73\ 3^{ab}$	1.0	.03	1.2
FCR	37	1.77^{a}	1.68^{c}	$1\ 68^{c}$	1.69^{bc}	1.69^{bc}	1.70^{b}	.6	.001	02
	45	2.02^{a}	1.94^{cd}	1.93^{d}	$1\ 95^{cd}$	1.98^{bc}	$2\ 00^{ab}$	1.3	.001	.04
Litter dry matter, %	37	67 1	68.4	69 1	66 3	69 6	71 9	6.5	.37	n.s.
Foot pad lesions, %	45	$11\ 0^{a}$	0.1^{b}	1.9^{b}	2.4^{b}	2.5^{b}	0.2^{b}	65.9	.002	7.0

[1] CV, Coefficient of variation
p<, the probability that difference(s) is caused by random.
LSD, Least significant difference for p<.01.
In each line, means with common superscript are not significantly (p<0.001) different from each other as analyzed by LSD procedure

counts of Cp in samples taken from the processing line after slaughter were higher than during the experimental period.

In both experiments all supplements significantly improved bird performance considering body weight, carcass yield, FCR, and foot health. In Expt. 2 carcass yield of birds fed growth promoters avilamycin or avoparcin was significantly higher compared with birds fed anticoccidials.

In Expt. 1 the addition of maduramicin to diets with avoparcin or avilamycin, in comparison with the substances alone, significantly decreased body weights and impaired FCR as

Table 7 Broiler chicken production performance in experiment 2

	Age, days	Control	Antibiotics: Avoparcin	Antibiotics: Avilamycin	Anticoccidials: Monensin	Anticoccidials: Narasin	Statistical analyses[1]: CV, %	p<	LSD
Mortality, %	38	5.3	4.3	3.8	4.1	4.4	20.3	.59	n.s
	43	6.1	4.8	4.6	4.7	5.3	20.3	.37	n.s.
Body weight, g	38	1887[b]	2147[a]	2101[a]	2106[a]	2101[a]	1.7	.001	53
	43	2174[c]	2398[a]	2355[ab]	2356[ab]	2333[b]	1.6	.001	44
Carcass yield, %	43	71.2[c]	72.8[a]	72 6[a]	72.0[b]	71.9[b]	0.6	.001	0.6
FCR	38	1.79[c]	1.72[b]	1.74[a]	1.72[b]	1.75[a]	0.8	.001	0.02
	43	1.95[c]	1.89[a]	1.90[a]	1.90[a]	1.93[b]	0.8	.001	0.02
Litter dry matter, %	35	66.8	66.9	67 2	71.8	68 1	5.0	.26	n.s.
Foot pad lesions, %	43	8.5[b]	2.8[a]	3.4[a]	2.3[a]	2.8[a]	37.0	03	4.0

[1] CV, Coefficient of variation.
p<, the probability that difference(s) is caused by random.
LSD, Least significant difference for p< 05.
n.s. = not significant.
In each line, means with common superscript are not significantly (p<0.05) different from each other as analyzed by LSD procedure.

measured at 37 and 45 days of age. The comparison of the combinations avoparcin/maduramicin and avoparcin/monensin revealed better FCR with maduramicin than with monensin as measured on day 45. The litter was more wet in pens with chickens fed maduramicin in comparison with monensin.

The addition of monensin to a diet with avoparcin significantly impaired FCR and decreased body weight as measured on day 45 in comparison with avoparcin alone.

Discussion

The results confirmed the expected growth promoting effect of the antibacterial drugs giving 6.8% higher weights at 37 days of age, on an average in Expt. 1. Similarly FCR was improved by about 5% and carcass yield increased by about 1 percent unit. Corresponding improvements in Expt. 2 were 12.5, 3.4 and 1.5, respectively.

The data also show that the ionophores monensin and narasin induced growth promoting effects similar to the tested growth promoters which may be attributed to their antibacterial effects. No additive effect was seen by combining growth promoter and ionophore anticoccidials.

The present results are somewhat contradictory to *Wang & Davidson* (1992). These authors compared efficacy of ionophore/growth promoter combinations on birds challenged with NE. The ionophores maduramicin, monensin or salinomycin alone did not reduce the incidence of NE, and only salinomycin significantly improved weight gains in comparison with the infected control. Addition of anyone of the growth promoters, avoparcin, virginiamycin or zinkbacitracin significantly reduced NE mortality and improved weight gains which were comparable to the uninfected, unmedicated control. There may be several reasons for this discrepancy between the work of *Wang & Davidson* (1992) and the present data such as differences in infection pressure, differences in activities of

substances used, and differences in bacteria (Cp) resistance pattern.

The number of Cp positive birds and the counts of Cp were higher in control birds than in the birds fed any of the test substances with reservation for the data day 29 in Expt. 2. As could be expected the number of Cp positive birds increased when the drugs were withdrawn before slaughter in Expt. 2. We have no explanation, however, why the caecal counts of the birds from which the anticoccidials have been withdrawn (Expt. 2) are higher than in the Control groups. It might be a result of an immunisation of the Control birds due to a higher exposure of Cp during the rearing, or the conditions of the intestinal environment after the influence of the medication may favour multiplication of Cp if present. Also in Expt. 1 in which the growth promoters were given all the time, there was an increase in the caecal content of Cp in samples taken from the processing line which may be attributed to the increased stress to the birds during handling in connection with slaughter and a longer time from killing to sampling. The Cp counts are in accordance with *Hofshagen & Kaldhusdal* (1992). Feeding diets without drug supplements, these authors found a peak in intestinal Cp counts at about 3 weeks of age. The Cp counts among birds fed avoparcin increased substantially within a few days following withdrawal of antibiotic supplementation, and reached a level similar to birds that had not been given the feed antibiotic.

The incidence of NE was very low, 0.2% (13 cases) in Expt. 1, and 2 cases in Expt. 2. All cases except one occurred in the control groups. From this no clear conclusions regarding preventive effect of the test substances could be drawn, but the effects on the intestinal growth of Cp is supposed to be related to the risk of the occurrence of NE. However, possible differences in the mode of action of the test substances related to differences in Cp strains to produce extracellular substances (toxins) also have to be considered.

Numerous reports have shown that antibiotics reduce the thickening of the intestinal wall (*Jukes et al.* 1956, *Stutz et al.* 1983a, 1983b, *Stutz & Lawton* 1984). This effect has been associated with suggested changes in the intestinal microflora. In practice these effects may affect the processing yield which was demonstrated in the present investigation. The higher processing yield of birds fed antibiotics compared with anticoccidials in Expt. 2 may be due to differences in effects on the intestinal microflora and intestinal wall thickness.

The beneficial effect on foot health by the tested additives may be related to effects on excreta consistency and/or dry matter content favouring better litter conditions.

The EU-commission banned the use of avoparcin in poultry diets in April 1997 due to the risk that use of avoparcin in feed may lead to development of bacterial resistance against vancomycin, an antibiotic used in human medicine (*Bates et al.* 1994, *Aarestrup et al.* 1996, *Howarth & Poulter* 1996). This limits the access to feed antibiotics for the poultry industry. Since the occurrence of resistant bacteria is a growing world wide problem, a general ban of feed antibiotics may follow in the future. Therefore precautions and preparations are necessary to face this situation. In Sweden feed antibiotics have been banned since 1987, and ionophore anticoccidials (mainly narasin) have been used as single supplements without any obvious effects on production performance. However, at the same time precautions have been taken considering feed hygiene, feed composition, and management conditions.

Conclusions

The results of the present experiments show that, during high hygienic standard, broilers

may be raised without growth promoters without any detrimental effect on production performance as long as ionophore anticoccidials are used. Further research is needed to solve the problems which probably will arise if the anticoccidials are replaced by vaccines.

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Sammanfattning

Inverkan av tillväxtstimulerande foderantibiotika och kokcidiostatika på produktionsresultat och tillväxt av Clostridium perfringens i blindtarm hos slaktkycklingar

Tillväxtstimulerande foderantibiotika, avoparcin och avilamycin, samt jonofora kokcidiostater maduramicin, narasin och monensin studerades i 2 försök med slaktkycklingar avseende inverkan på produktionsresultat och tillväxt av *Clostridium perfringens* (Cp) i blindtarm. Substanserna tillsattes foderblandningen antingen enskilt eller i vissa kombinationer. Inga tecken på sjukdom eller tarmförändringar orsakade av kokcidier observerades. Alla testsubstanserna hade en antibakteriell effekt på Cp och var signifikant tillväxtförbättrande. Resultaten visar, vad produktionsresultaten beträffar, att under goda hygienförhållanden kan foderantibiotika undvaras om foderblandningen tillsätts ett kokcidiostatikum med antibakteriell effekt

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