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Effect of monensin on the crop microflora of broiler chickens

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Summary — Experiments were undertaken to evaluate the effect of monensin on both the microorganisms and their metabolite production in the crop of 3-week-old broiler chickens. The addition of monensin to the feed (100 mg/kg) significantly ($P < 0.05$) lowered the number of lactobacilli in the crop and decreased the concentration of lactate, acetate, succinate and ethanol in both in vivo and in vitro incubations of the crop contents. The decreased acid production increased the pH of the digesta and also the pH of the monensin-supplemented cultures ($P < 0.01$). The decrease in the number of lactobacilli was accompanied by a concomitant proliferation of coliform bacteria and enterococci. The lactobacilli counts returned to the pretreatment level within 5 days of monensin feeding. The counts of coliforms and enterococci, however, remained elevated. The concentration of lactate in the crop was lower and the pH higher in the monensin-fed chickens than in the control chickens ($P < 0.005$). The microbial metabolite production was lower in all the cultures supplemented with monensin, both in the control incubations and in those inoculated by the crop contents of monensin-fed chickens ($P < 0.01$). The effect of monensin in vitro ceased after 10 days of monensin feeding to the inoculi donors. In the presence of monensin, the crop environment was more suitable to the proliferation of coliforms, which might be a factor in the increased incidence of nonspecific septicemia.

chicken / crop microflora / monensin

Résumé — L'influence de la monensine sur la microflore du jabot de poulets. *Nous avons étudié l'influence de la monensine sur les microorganismes et la composition des métabolites microbiens dans le jabot de poulets agés de 3 semaines. La diminution du nombre de lactobacilles dans le jabot ainsi que celle de la concentration en lactate, acétate, succinate, alcool éthylique et propionate in vivo et durant les incubations in vitro du contenu de jabots par l'addition de la monensine ont été significatives (*$p < 0,05$*). L'addition de la monensine a entraîné – en raison de la diminution de la production des acides – une augmentation du pH du contenu de jabot ainsi que des cultures (in vitro) –* $p < 0,01$*. L'inhibition des lactobacilles s'est accompagnée d'une augmentation du nombre des bactéries coliformes et des enterocoques. Après 5 jours de distribution de la monensine le nombre de lactobacilles*

a retrouvé sa valeur initiale tandis que les populations de coliformes et d'enterocoques sont restées élevées. On a constaté ($p < 0,01$) une diminution des métabolites microbiens dans toutes les cultures enrichies en monensine. L'influence de la monensine sur les cultures in vitro cesse après 10 jours de distribution aux poulets donneurs. En présence de monensine, le contenu du jabot constitue un milieu plus favorable à la prolifération des coliformes, ce qui peut augmenter l'incidence des septicemies non spécifiques.

poulet / microflore du jabot / monensine

INTRODUCTION

The crop is the first segment of the chicken digestive tract. In this region the ingested food is stored and acidified as a result of the metabolic activity of symbiotic microorganisms located there. The crop microflora is less complex than that inhabiting the downstream intestinal tract; nevertheless, it provides an effective barrier against colonization by human foodborne pathogens. The predominant organisms in the crop are lactobacilli (Sarra et al, 1992). Crop bacteria are presumably susceptible to ionophore antibiotics (monensin, salinomycin, lasalocid, narasin), which are widely used to control coccidiosis in poultry. The aim of our study was to determine the effect of monensin on the counts of several groups of bacteria and on the composition of microbial metabolites in the chicken crop. Various authors have examined the efficacy of monensin against *Eimeria* sp (Bafundo and Jeffers, 1990; Salisch and Shakshouk, 1990) and the effect of monensin on the performance of broiler chickens (Parson and Baker, 1982; Christmas and Harms, 1984; Harms et al, 1989). High concentrations of monensin in broiler feeds (140–150 mg/kg) depresses the weight gain (Bartov, 1987) and can sometimes be toxic (Braunius, 1989). In ruminants, monensin alters the rumen fermentation pattern towards a greater production of propionate and lower amounts of acetate, butyrate, lactate and methane (Chalupa et al, 1980; Dennis et al, 1981). Among the rumen bacteria, monensin suppressed the growth of the Gram-positive species (streptococci, lactobacilli) and those species that stain Gram-negatively, but have Gram-positive structures in their cell walls (butyrivibrios, ruminococci). To our knowledge, no similar studies have been performed in poultry, except some simple antimicrobial susceptibility tests in chicken lactobacilli (Dutta and Devriese, 1981, 1992; Rada et al, 1994).

MATERIALS AND METHODS

Chicken

Forty 3-week-old broiler chickens (Ross) were fed a commercial BR 2 feed mixture containing ground maize (42%), ground wheat (26%), extracted soybean meal (25%), meat and bone meal (2%), fish meal (1%), dried yeasts (1%) and a vitamin-mineral supplement (3%). A control group (ten birds) received no monensin. The chickens in the treated groups (ten birds per group) received monensin (Elanco) in the feed mixture (100 mg/kg) at a recommended level for 2, 5 and 10 days.

Enumeration of bacteria

Four chickens from each group were killed by cervical dislocation. The contents of the crop were collected aseptically, serially diluted in sterile Wilkins-Chalgren anaerobic broth and plated on Rogosa agar, Endo agar and Slanetz-Bartley agar to enumerate the lactobacilli, coliform bacteria and enterococci, respectively. The bacteriological media were purchased from Oxoid. The plates were incubated aerobically at 37 °C for

24 h (coliforms), 48 h (enterococci) or anaerobically at 37 °C under CO_2/H_2 atmosphere for 48 h (lactobacilli).

In vitro incubations

The BR 2 feed mixture (73 g) with monensin (100 mg/kg) was mixed with water (147 mL) and inoculated by adding 2.2 g of the crop content. The remaining chickens were killed and used for this purpose. The inoculated cultures (four per group) were incubated in 300 ml serum bottles under CO_2 atmosphere at 42 °C for 150 min. Another four incubations inoculated by the crop contents contained no monensin. After incubation, the pH of the cultures was measured and the lactobacilli, coliforms and enterococci were measured as described earlier. The culture samples were frozen and stored at –20 °C for further analyses. The significance of differences was evaluated by the *t*-test.

Analysis

All samples were centrifuged (15.10^3 g for 30 min) and the supernatant used for analyses. Volatile fatty acids and ethanol were determined in acidified chymus by gas chromatography using 1.6 m columns with 4% Carbowax 20 M on Carbopack B–DA (Supelco). A temperature program (80–120 °C) was employed to permit complete separation into individual compounds. Lactic and succinic acids were esterified by methanol according to Bricknell et al (1979) and determined on the Megabore DB–FFAP column 30 m x 0.53 mm (J & W, Folson, USA) with a precolumn 1 m x 0.53 mm, using a temperature program (110–175 °C) and crotonic acid as an internal standard. The significance of differences was evaluated by the *t*-test.

RESULTS

Lactobacilli were the most numerous microorganisms present in the crop of all four chicken groups (table I). The lactobacilli counts were significantly ($P < 0.05$) lower in the crop of chickens fed monensin for 2 days. The difference in the lactobacilli counts, however, disappeared when monensin was fed to the chickens for 5 days. Counts of coliform bacteria and enterococci were higher in all monensin-fed chickens. The concentration of lactate was significantly ($P < 0.005$) lower in the crop of monensin-fed chickens (table II). The lower concentrations of acidic metabolites in the treated chickens were more pronounced in chickens fed on monensin for 2 days than in those fed monensin for 10 days.

Monensin significantly ($P < 0.01$) decreased the lactobacilli counts in the in vitro incubations of the crop contents taken from the control chickens, but had no effect on lactobacilli counts in the incubations of the crop contents of monensin-fed chickens (table III). The numbers of coliform bacteria were significantly ($P < 0.01$) increased

Table I. Bacteria counts[1] in the crop of control and monensin-fed broiler chickens.

Treatment	*Lactobacilli*	*Coliforms*	*Enterococci*
Control	8.80 ± 0.44	5.54 ± 0.42	4.64 ± 0.10
Monensin (2 days)	7.96 ± 0.48*	6.64 ± 0.43*	5.36 ± 0.66
Monensin (5 days)	8.56 ± 0.30	6.48 ± 0.26*	6.91 ± 0.33*
Monensin (10 days)	8.54 ± 0.05	6.05 ± 0.13	5.37 ± 0.50*

[1] Log_{10} cfu/g of digesta (mean values ± SD); * significantly different from control chickens ($P < 0.05$).

Table II. Microbial metabolite concentrations[1] and pH values in the crop of control and monensin-fed chickens.

Parameter	*Treatment of chickens*		
	Control	*Monensin (2 days)*	*Monensin (10 days)*
pH	4.29 ± 0.07	5.10 ± 0.04*	4.61 ± 0.08*
Lactate	188.0 ± 19.7	47.3 ± 13.0*	93.6 ± 29.2*
Acetate	33.2 ± 7.0	17.1 ± 9.0	25.9 ± 1.7
Propionate	0.5 ± 0.2	0.4 ± 0.2	0.5 ± 0.1
Succinate	8.1 ± 2.7	4.5 ± 3.2	3.6 ± 0.6
Ethanol	2.4 ± 1.2	1.0 ± 0.6	3.8 ± 3.8

[1] mM (mean values ± SD); * significantly different from the control ($P < 0.005$).

Table III. Bacteria counts[1] in in vitro cultures inoculated by the crop contents of control and monensin-fed chickens.

Treatment of chickens	*Monensin addition to cultures (μg/g)*	*Lactobacilli*	*Coliforms*	*Enterococci*
Control	0.0	8.73 ± 0.12	5.30 ± 0.14	4.47 ± 0.32
Control	33.3	7.27 ± 0.22*	6.14 ± 0.23*	3.90 ± 0.27
Monensin (2 days)	33.3	8.54 ± 0.12	6.68 ± 0.34*	5.56 ± 0.14*
Monensin (5 days)	33.3	8.35 ± 0.19	6.38 ± 0.36*	6.41 ± 0.11*
Monensin (10 days)	33.3	8.69 ± 0.13	6.32 ± 0.14*	5.13 ± 0.42*

[1] Log_{10} cfu/g (mean values ± SD); * significantly different from monensin-free control chickens ($P < 0.05$).

in all monensin-containing cultures, in comparison with monensin-free controls. The number of enterococci were lower when monensin was added to the crop content cultures of the control chickens. The same bacteria proliferated in cultures of crop contents taken from monensin-fed chickens.

Lactate was the principal microbial metabolite in all crop content incubations (table IV). Monensin lowered the lactate concentration ($P < 0.01$) and also concentrations of the other metabolites (acetate, succinate and ethanol). The effect of monensin on the production of lactate disappeared when the cultures were inoculated by the crop contents of chickens fed monensin for 10 days.

DISCUSSION

Monensin has long been widely employed as a feed additive to control coccidiosis, which is endemic in poultry (Shumand and Callender, 1968). In the chicken digestive tract, monensin is partially absorbed and

Table IV. Microbial metabolite concentrations[1] and pH values in in vitro cultures inoculated by the crop contents of control and monensin-fed chickens.

Culture parameter	*Treatment of chickens*				
	Control	*Control*	*Monensin (2 days)*	*Monensin (5 days)*	*Monensin (10 days)*
Monensin (μg/g)	0.0	33.3	33.3	33.3	33.3
pH	4.38 ± 0.06	5.38 ± 0.02*	4.93 ± 0.13*	4.92 ± 0.09*	4.89 ± 0.03*
Lactate	105.7 ± 15.7	14.1 ± 3.2*	41.5 ± 13.2*	27.2 ± 3.5*	118.5 ± 6.6
Acetate	19.7 ± 2.0	5.3 ± 0.5*	15.3 ± 3.5	10.9 ± 0.7*	32.7 ± 1.6*
Propionate	1.0 ± 0.1	1.1 ± 0.1	1.2 ± 0.2	1.1 ± 0.1	0.5 ± 0.1*
Succinate	7.3 ± 0.9	1.9 ± 0.5*	3.6 ± 1.1*	2.6 ± 0.2*	7.3 ± 1.2
Ethanol	2.1 ± 0.3	1.2 ± 0.1*	1.1 ± 0.4*	1.1 ± 0.2*	1.3 ± 0.4

[1] mM (mean values ± SD); * significantly different from monensin-free control chickens ($P < 0.01$).

metabolized (Davison, 1984). However, its concentration in the digesta must be high enough to influence bacterial growth; for this reason, monensin is added to feed mixtures at 100 mg/kg. The most numerous microorganisms in the upper digestive tract of chickens are lactobacilli (Smith, 1965). In the crop, lactobacilli acidify the swallowed food to a pH of 4–5. The acidity causes the growth of less aciduric organisms, including pathogens, to be suppressed. The level of susceptibility of poultry lactobacilli to monensin, found in agar dilution tests, varied from 0.25 to 6 μg/mL (Dutta and Devriese, 1981). The susceptibility of lactobacilli to monensin determined in laboratory tests differs from that existing in in vivo conditions. Rada et al (1994) found that poultry lactobacilli strains, inhibited in liquid media with glucose by monensin at 2 μg/mL, grew in wetted concentrate containing 100 mg of monensin per kg. The reason probably lies in the presence of glycocalyx, which is synthesized in an environment with solid particles, but which is lost in laboratory cultures with soluble substrates (Costerton et al, 1978). It followed from our data, that the addition of monensin to the chicken feed lowered numbers of lactobacilli in the crop and also the concentration of microbial metabolites. The decrease in lactobacilli counts was accompanied by the concomitant proliferation of coliforms and enterococci. Similar shifts in microbial population were observed in the rumen of ionophore-fed calves (Kobayashi et al, 1989). It is shown in table I that the crop lactobacilli adapted somewhat to the presence of monensin within 5 days. A decrease of acidic metabolite concentrations and an increase in the number of coliforms and enterococci in vivo was more stable. The return to control levels of these parameters was not observed in chickens fed monensin for 10 days (tables I and II). Similar conclusions can be drawn from the in vitro experiments, with the exception of the microbial metabolites production. These achieved the same level as the control chickens after 10 days of monensin feeding (table IV).

The results presented in this paper demonstrated that the use of monensin in chicken feeding interfered with the microbial growth and metabolism in the crop. In the presence of monensin, the crop environment was more suitable for the proliferation of coliforms, which might increase the incidence of nonspecific septicemia in poultry.

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