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Comparison of control methods for coccidiosis in native Spanish 'Castellana Negra' chickens

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Abstract

Coccidiosis is a disease responsible for serious economic losses in the poultry industry. This paper compares the effect of coccidiosis infection in a population of experimentally infected Castellana Negra chickens previously administered the ionophorous antibiotic monensin (Treatment 1), Alquernat Zyox®, a herb-based product (Treatment 2), or a live vaccine based on oocystes selected for precocity (Treatment 3). Fifty birds per treatment were housed in captivity and weighed individually once every two weeks. At nine weeks they were infected with pathogenic oocysts of *Eimeria tenella, E. acervulina* and *E. maxima*. No significant differences (P<0.05) were seen in body weight between the birds in the three treatment groups after week 10. The average daily weight gain of the Alquernat Zycox®-treated and vaccinated birds was similar over the entire experimental period, and more regular than that of the monensin-treated birds. The number of oocysts eliminated in the faeces and the degree of intestinal injury caused were analysed at 10, 11 and 12 weeks. The vaccine-treated birds shed a smaller number of oocysts in their faeces at 11 and 12 weeks than did those treated with monensin or Alquernat Zycox® (P<0.001). At 11 and 12 weeks the vaccine- and Alquernat Zycox®-treated birds showed significantly (P<0.05) less intestinal injury than the monensin-treated birds.

Additional key words: Alquernat Zycox®; *Eimeria*; monensin; oocysts; vaccine.

Resumen

Comparación de diferentes métodos de control de coccidiosis en gallinas de la raza autóctona Castellana Negra

La coccidiosis es una parasitosis con gran incidencia económica en la industria avícola. En el presente trabajo se comparan los efectos tras la infección experimental por coccidios en una población de pollos de raza Castellana Negra, utilizando el antibiótico ionóforo monensina (Tratamiento 1), un producto natural como es Alquernat Zycox® (Tratamiento 2) y una vacuna de ooquistes atenuados por precocidad (Tratamiento 3). Un total de 50 aves por grupo se alojaron en parques en cautividad, las cuales se pesaron individualmente cada 2 semanas. A las 9 semanas se infectaron experimentalmente con ooquistes patógenos de *Eimeria tenella, E. acervulina* y *E. maxima*. No hubo diferencias significativas entre el peso corporal de los pollos de los distintos grupos en la semana 10. La ganancia media de peso diario de los pollos tratados con Alquernat Zycox® y los vacunados fueron similares durante todo el periodo experimental. Es más, su crecimiento fue más regular que el de los pollos tratados con monensina. A las 10, 11 y 12 semanas se analizó el número de ooquistes eliminados en las heces y el grado de lesiones intestinales. Las aves vacunadas eliminaron una cantidad inferior de ooquistes que las tratadas con monensina y también presentaron menor número de ooquistes en las heces a las 11 y 12 semanas postinfección que los tratados con Alquernat Zycox® (P<0,001). El grado de lesiones a las 11 y 12 semanas fue significativamente inferior (P<0,05) en las tratadas con la vacuna y con Alquernat Zycox® que en las tratadas con monensina.

Palabras clave adicionales: Alquernat Zycox®; *Eimeria*; monensina; ooquistes; vacuna.

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Abbreviations used: ADGW (average daily gain in weight), EC (European Commission), PCA (pharmacological coccidiostatic agent).

Introduction

Coccidiosis, a disease of poultry caused by *Eimeria* spp., is responsible for serious economic losses - up to €1500 million annually worldwide (Escoda, 2003). Several anticoccidial drugs have been developed to help control this problem. Monensin, an ionophorous antibiotic produced by *Streptomyces cinnamonensis*, has long been the most used anticoccidial agent in the poultry industry. However, despite the warnings made (Ruff, 1993), resistance to almost all ionophorous antibiotics has now appeared (Chapman, 1993) (resistance is probably the single most important factor in the demise of the effectiveness of these drugs [Li *et al.*, 2005]), there are concerns about drug residues in poultry products, and there is a strong consumer desire to do away with drugs in animal feeds. Recent norms (EC Directives 2092/1991 [OJ, 1991] and 1804/1999 [OJ, 1999]) require that poultry producers dispense with the use of certain drugs when feeding their birds.

The pressing need to move away from the chemotherapeutic control of coccidiosis has led to increased interest in vaccination. Many attenuated anticoccidial vaccines are currently in use (Li *et al.*, 2005). Various herb extracts are also known to be effective against parasites such as the malaria parasite, amoebae, trichomonads, arthropods and helminths (Ou-Yang *et al*., 1990; Quan, 1990). Youn and Noh (2001) report on the efficacy of extracts of *Bupleurum chinese*, *Pulsatilla koreana*, *P. aviculare*, *Ulmus macrocarpa*, *Sophora japonica*, *Torilis nucifera*, *Quisqualis indica*, *Sophora flavescens* and *Sophora acutum* as anticoccidial agents.

The present work compares the effects of coccidiosis in a population of experimentally infected Castellana Negra chickens previously administered the ionophorous antibiotic monensin, the herb extract Alquernat Zycox®, or a vaccine.

Material and methods

The experimental birds used in this work were 1 day-old males of the Castellana Negra chicken breed (a breed native to Spain). These were assigned to three treatment groups of 50 chicks each and housed in adjacent pens over litter beds (4 birds m^{-2}) for 12 weeks.

Treatment 1 birds were fed an ecological feed based on cereal grains, products of oleaginous seeds, oils and fats and minerals (see Table 1) supplemented with monensin (50 ppm). Treatment 2 birds were administered Alquernat Zycox® (BIOVET S.A., Tarragona, Spain), a natural coccidiostatic agent (1000 ppm) made from the extracts of *Hollarrhena antidysenterica* (30%), *Berberis aristata* (30%), *Allium sativum* (15%) and *Embelia ribes* (15%) the components of which include phenols, essential oils, phytoproteins and a large quantity of sulphated amino acids and glycosides. Treatment 3 birds received an attenuated vaccine (administered orally on day 1 of life) prepared with a mixture of precocious strains of *E. acervulina*, *E. maxima* and *E. tenella* (manufactured and administered by the Faculty of Veterinary Medicine, University of Zaragoza, Spain). Treatment 2 and 3 birds were fed the same feed provided to the Treatment 1 birds, but without the antibiotic. Feed was provided *ad libitum* to all groups. When the chickens were 9 weeks old, they were experimentally infected with pathogenic oocysts derived from field samples. The inoculum contained 10,000 *E. tenella* oocysts, 100,000 *E. acervulina* oocysts, and 15,000 *E. maxima* oocysts.

Table 1. Estimated nutrient content of the diet fed during the trial

Nutrient contents	**%**
Crude protein	19
Ether extract	6.4
Methionine	0.45
Gross fibre	3.6
Ash	5.1
Starch	30
Ca	0.85
P	0.65
Additives	
Vitamin A (IU)[a]	10,000
Vitamin D3 (IU)	2,000
Vitamin E (mg)	22
Cu (mg)	5

[a] IU: international units

At 10, 11 and 12 weeks, 10 chickens from each group were sacrificed and the degree of intestinal injury they suffered determined. Briefly, samples of duodenum, the middle third of the intestine and the caeca were examined by light microscopy and the injury they suffered classified on the 4 point scale defined by Johnson and Reid (1970): 0 = normal, 1 = mild injury, 2 = moderate injury, 3 = serious injury, 4 = very serious injury with death. Since the different species of *Eimeria* affect dif-

ferent areas of the intestine and induce lesions with different characteristics (*E. acervulina* affects the upper part of the small intestine, inducing white plaques; *E. maxima* affects the mid small intestine, inducing small, white, round lesions; *E. tenella* affects the caeca, inducing thickening and haemorrhaging), those caused by each species in the inoculum were recorded.

The mean number of oocysts per gram of faeces for each treatment group at weeks 10, 11 and 12 was determined using the McMaster flotation method (Gordon and Whitlock, 1939), as modified by Whitlock (1948).

The body weight of each bird in each treatment group was measured every two weeks, from the 4th until the 12th week (the end of the experimental period).

Differences between means were analysed using the Student *t* test. All calculations were performed using SPSS v.10.0 software for Windows (SPSS Inc., 1999). Significance was set at P=0.05 for all variables except oocyste number (P=0.01).

This study was approved by the Ethics Committee for Animal Experimentation of the College of Agricultural Engineers, University of Valladolid (Soria Campus), Spain**.**

Results

No deaths were recorded in any of the groups during the course of the experiment. No significant differences were seen in the mean body weight of the chickens of any treatment group at any time (Table 2). The Treatment 2 and 3 birds showed a more regular average daily weight gain (ADGW) than the Treatment 1 birds. In fact, the ADGW of the monensin-treated birds was lower than that of the other groups until week 10, when it became higher. However, in week 11 it fell once again to below the values recorded for the Treatment 2 and 3 birds, only to rise to match them in week 12.

Table 3 shows that in weeks 10, 11 and 12 the vaccinated animals eliminated significantly fewer oocysts in their faeces than those treated with monensin (P<0.001). Indeed, throughout the experiment, the largest number of oocysts in faeces was recorded for the monensin-treated birds. No significant differences were found between the number of oocysts in the faeces of the Alquernat Zycox®-treated and vaccinated birds in week 10; in weeks 11 and 12 the Alquernat Zycox®-treated birds shed significantly more oocysts.

Table 4 shows the lesion severity results for the birds at 10, 11 and 12 weeks considering all the species of *Eimeria* as a whole. The vaccinated birds showed the least serious injuries at all inspection times; the birds of the other two treatment groups suffered significantly more damage.

Table 4 shows the lesion severity suffered by the three groups of birds taking into account the different species of *Eimeria* separately. At week 10, there were significantly fewer (P<0.05) lesions caused by *E. tenella* and *E. maxima* in the vaccinated birds than in the other treatment groups. No differences were seen between the treatment groups with respect to damage caused by *E. acervulina*. In week 11, the damage caused by *E. tenella* and *E. maxima* was the same in the vaccinated and Alquernat Zycox®-treated birds. Also at 11 weeks, fewer *E. Acervulina*-induced lesions were seen in the vaccinated birds than in the other treatment

Table 2. Mean ± standard deviation weight and average daily gain (ADGW) for the three treatment groups

Week	Monensin		Alquernat Zyox®		Vaccine	
	Weight (g)	ADGW (g)	Weight (g)	ADGW (g)	Weight (g)	ADGW (g)
4	235.28^{a} ± 30.4		225.35^{a} ± 23.8		257.75^{a} ± 40.3	
6	476.61^{a} ± 48.2	17.23^{1} ± 2.1	447.83^{a} ± 36.7	15.89^{1} ± 2.2	470.47^{a} ± 42.3	15.19^{1} ± 2.5
8	802.23^{a} ± 63.5	23.25^{1} ± 3.2	774.53^{a} ± 65.2	23.33^{1} ± 2.9	796.32^{a} ± 67.2	23.27^{1} ± 3.0
9 (infection)	832.22^{a} ± 76.4	7.49^{2} ± 0.9	802.4^{a} ± 69.2	6.96^{2} ± 1.1	852.12^{a} ± 77.8	13.95^{1} ± 2.2
10	1126.0^{a} ± 87.2	29.38^{1} ± 2.4	995.58^{a} ± 90.5	19.32^{2} ± 2.4	1040.24^{a} ± 88.3	18.81^{2} ± 2.1
11	1140.2^{a} ± 90.7	2.02^{2} ± 0.2	1163.4^{a} ± 93.2	23.97^{1} ± 2.3	1219.3^{a} ± 97.1	25.58^{1} ± 3.1
12	1283.9^{a} ± 97.7	20.5^{1} ± 3.1	1334.6^{a} ± 101.2	24.45^{1} ± 3.2	1360.55^{a} ± 92.3	24.46^{1} ± 3.5

Means followed by different letters (weight) or numbers (ADGW) in the same row are significantly different (P<0.05)

Table 3. Mean number of oocysts per gram of faeces

Week	Monensin	Alquernat Zyox®	Vaccine
10	185,484[a]	44,672[b]	34,851[b]
11	246,216[a]	50,495[b]	17,411[c]
12	99,106[a]	19,074[b]	9,089[c]

Means followed by different letters in the same row are significantly different (P<0.001).

groups. In week 12, the damage caused by *E. tenella* and *E. maxima* was significantly greater in the birds treated with monensin than in the other treatment groups. Finally, at 12 weeks, no differences were observed between the groups in terms of *E. Acervulina*-induced damage.

Discussion

The Castellana Negra breed, which belongs to the class of slow-growing chicken lines, is a native of Spain currently classified as endangered. However, it is extremely hardy and disease-resistant, and is therefore a potential meat producer of some interest.

Monensin works by modifying the passage of Na^+ and K^+ through the cell membrane, creating an osmotic imbalance that finally destroys the parasite. In contrast, Alquernat Zycox® affords protection by inducing the optimum physiological state of the mucosa and improving local immunity. The vaccine used provides immunological protection that lasts throughout life if administered soon after hatching.

Pharmacological agents are the most common choice for controlling coccidiosis in poultry since they can be added to feed. However, the European Commission (EC) has opted for restricting the use of pharmacological coccidiostatic agents (PCAs); for example, EC Directive 2205/2001 (OJ, 2001) bans the use of amprolium, amprolium-ethopabate, dimetridazole, methylchlorpindol, nicarbazine and methylchlorpindol/methylbenzoquate. The possibility of PCAs losing their category as feed additives has led to the search for other control methods (among which vaccines and plant derivatives appear to be the most effective). The substitution of such products could avoid their residues accumulating in meat as well as the interaction of these products with others commonly used in the poultry industry, such as antibiotics or antioxidants, etc. (Dowling, 1992).

Vaccines containing attenuated *Eimeria* strains selected for precocity have been used extensively for several years, and are associated with production results similar to those achieved with PCAs (Shirley *et al.*, 1995). Plant derivatives have not been tested on a large scale in the poultry industry, and their accumulation in poultry meat and the possible organoleptic changes they may cause are still to be determined. If good results are obtained,

Table 4. Mean intestinal damage (Johnson and Reid [1970]) caused by infection in each group of chickens. Results are shown for each *Eimeria* species separately and as a whole

Week	Species	Monensin	Alquernat®	Vaccine
10	*E. tenella*	2.75[a]	2.25[ab]	1.25[b]
	E. acervulina	2.25[a]	1.75[a]	1.5[a]
	E. maxima	2.5[a]	1.5[ab]	0.75[b]
	Eimeria in bulk	2.6[a]	2.0[ab]	1.4[b]
11	*E. tenella*	3.25[a]	1.5[b]	1.25[b]
	E. acervulina	2.0[a]	1.25[ab]	0.5[b]
	E. maxima	2.25 [a]	0.75[b]	0.5[b]
	Eimeria in bulk	3.2[a]	1.8[b]	1.4[b]
12	*E. tenella*	2.5[a]	1.25[b]	1.25[b]
	E. acervulina	1.75[a]	1.0[a]	0.5[a]
	E. maxima	2.25[a]	0.5[b]	0.75[b]
	Eimeria in bulk	2.4[a]	1.4[b]	1.2[b]

Means followed by different letters in the same row are significantly different (p<0.05). Injury suffered : 1 = light injury; 2 = moderate injury; 3 = serious injury; 4 = very serious injury with mortality

these could be easily used and would be cheaper than vaccines.

Allen *et al.* (1998) report that sources of fat containing high concentrations of n-3 fatty acids, such as flaxseed oil, reduce the lesions caused by the *E. tenella* but not those caused by *E. maxima*. Artemisinin, a naturally occurring endoperoxide from *Artemisia annua* —an effective anti-malarial agent— significantly reduces the intestinal damage caused by *E. tenella* when given at low levels as a feed additive. Diets supplemented with tocopherol (abundant in flaxseed) reduce mid-small intestinal lesion scores and improve weight gains when birds are infected with *E. maxima*. Betaine may act as an osmoprotectant, improving the integrity and function of the infected intestinal mucosa. Youn and Noh (2001) indicate that extracts of *S. flavescens*, *P. koreana*, *S. acutum*, *U. macrocarpa* and *Q. indica* are effective at reducing diarrhoea, intestinal damage and oocyste shedding, and increasing body weight gains.

Lee *et al.* (2007) reported that *E. acervulina*- and *E. tenella*-infected birds fed diets supplemented with Mito-Max (a commercial probiotic that contains *Pediococcus acidilactici* and *Saccharomyces boulardii*) shed fewer oocysts ($P<0.05$) than control infected chickens. In addition, chickens infected with *E. acervulina* fed a Mito-Max-supplemented diet showed significantly ($P<0.001$) higher serum *Eimeria*-specific antibodies counts.

Vermeulen *et al.* (2001) concluded that vaccination is the best long-term solution for the problems caused by these pathogens. However, improving the physiological and thus immunological fitness of birds via better hygiene management and using specific feed supplements could certainly complement this approach to disease control.

Guzman *et al.* (2003) reported that vaccines based on deactivated virulent *Eimeria* strains led to low oocyste numbers and high serum antibody titres for *E. acervulina* and *E. tenella*, while birds vaccinated with an attenuated vaccine showed the same only with respect to *E. maxima*. Using a multivalent, attenuated vaccine produced with ionophore-tolerant *Eimeria* strains, Li *et al.* (2005) found that the final mean weight of the vaccinated birds was significantly higher ($P < 0.05$) than that of controls. Working with two attenuated anti-coccidial vaccines, Ceruti *et al.* (2005) found maximum oocyste counts of 23,000 g^{-1} faeces at 28 days of age and 38,000 at 21 days respectively – more than that recorded for the present vaccinated birds at weeks 11 and 12, but similar to that recorded at week 10. Guo *et al.* (2005) reported that at 7 days post-infection, birds fed a diet supplemented with a plant extract (*Astragalus membranaceus*) had gained less weight than their vaccinated counterparts.

The production of ecological chickens is regulated by EC Directives 2092/1991 (OJ, 1991) and 1804/1999 (OJ, 1999), which prohibit the administration of pharmacological products. Therefore, coccidiosis can only be controlled via vaccines or plant derivatives registered as "Dietetic Complements". The present results, which confirm those obtained by other authors, show that vaccination or the administration of plant-extract coccidiostatic agents are effective means of fighting coccidiosis in poultry. These alternative treatments appear to be more effective than monensin treatment, possibly because the massive use of this drug has generated resistant strains (Chapman, 1993).

In conclusion, monensin was the least effective of the three treatments tested and was associated with significantly more serious intestinal lesions than seen with the other treatments. Except for small variations at certain times, treatment with Alquernat Zycox® or vaccination appeared to be equally effective.

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