VACCINATION ASSOCIATED TO A SALT MIX ADDED TO THE ORGANIC LITTER FOR COCCIDIOSIS PROFILAXIS IN POULTRY PRODUCTION

IMPIEGO DELLA VACCINAZIONE ABBINATA AD UNA MISCELA DI SALI COME ADDITIVO DELLA LETTIERA PER LA PER PROFILASSI DELLA COCCIDIOSI NEL POLLO

PAOLO MANI [1], MARCO BAGLIACCA [2],
MARCO FONTANELLI [3], GIACOMO ROSSI [4]

SUMMARY

CRG, a mineral mix used as litter pets or pets litter supplement, was preliminary tested, as pure litter for growing chickens resulting no toxic per contact or ingestion. CRG was then used, as supplement in a deep litter poultry house (0.5-1 Kg/m^2) on a colored low-growth-rate broiler, according to a 2*2 factorial experimental design (presence or absence of CRG and use or not use of a live attenuated vaccine for coccidiosis).

The following parameters were evaluated at 25, 33, 40 and 46 days: – birds, live weights, feed consumption metabolic profiles, oocyst counts on intestinal contents and histological controls: – litter, dry matter, gas production, oocysts (sporulated and not sporulated) and microbial counts.

Results showed that: CRG presence improved the live weight of 33 and 40 days old chicken and the dry matter of the litter at 40 days, decreased the protozoa counts in the litter and the percentage of sporulated oocysts. Vaccine reduced protozoa counts in gut and percentage of sporulated oocysts.

Key words: litter mineral premix, coccidiosis vaccination, broilers.

RIASSUNTO

Il CRG Solvay, una miscela di sali minerali usata come lettiera per animali d'affezione, è stato testato preliminarmente come lettiera pura per pulcini da 1 a 30 giorni di età, risultando non tossico (anche se ingerito o per contatto prolungato con la cute) ed è pertanto stato utilizzato in aggiunta alla lettiera organica (0,5-1 Kg/m^2) di un capannone di polli colorati a lento accrescimento. Lo schema sperimentale (fattoriale 2*2) preve-

[1] Dipartimento di Patologia Animale, Profilassi, Igiene degli Alimenti - Direttore Prof. Giovanni Braca.
[2] Dipartimento di Produzioni Animali - Direttore Prof. Paolo Verità.
[3] Collaboratore esterno.
[4] Dipartimento di Scienze Veterinarie, Università di Camerino - Direttore Prof. Ranieri Carlo.

deva la valutazione della presenza o assenza dell'integratore della lettiera (CRG) e l'uso o non uso di un vaccino vivo attenuato per le sette specie di Eimeria che parassitano il pollo domestico (PARACOX® - Schering S.p.A.).

Sono stati quindi misurati i seguenti parametri a 25, 33, 40 e 46 giorni: peso vivo, consumo alimento, profilo metabolico conteggio oocisti e rilievi anatomopatologici ed istologici sull'intestino degli animali; sostanza secca, produzione di ammoniaca, presenza di oocisti (sporulate e non sporulate) e carica microbica della lettiera.

I risultati hanno mostrato che la presenza del CRG ha migliorato l'accrescimento dei polli (33 giorni CRGno = 563 g; CRGsi = 673 g; 40 giorni CRGno = 716 g; CRGsi = 758 g) ha ridotto l'umidità della lettiera (40 giorni: CRGno = 58,7%; CRGsi = 27,7%) e diminuito la carica protozoaria e la percentuale di sporulazione delle oocisti dei coccidi (CRGno = 75,5%, CRGsi = 56,7%) nella lettiera. Nei polli vaccinati la carica protozoaria a livello enterico è risultata ridotta rispetto ai non vaccinati (53 giorni $2,31^{E+05}$ vs. $2,84^{E+05}$) e la percentuale di sporulazione delle oocisti presenti nelle feci inferiore (62,8%, *vs.* 70,0%). I risultati ottenuti dimostrano che la vaccinazione associata all'aggiunta di CRG alla lettiera organica, può essere un metodo efficace per tenere sotto controllo le coccidiosi, in alternativa ai metodi di profilassi farmacologica.

Parole chiave: vaccinazione coccidiosi, lettiera organica, CRG sale minerale, pollo da carne.

INTRODUCTION

Coccidiosis is a disease with a significant economic impact on the poultry industry, particularly in the early phases of breeding of broiler and roaster chickens in which a loss in weight gain and high mortality rate are described (Bafundo & Donovan, 1988). In addition the coccidial infection increases significantly the incidence of mortality rate by other pathogens as bacteria and viruses (Abrahmsson et al., 1998; Williams et al., 1999) and the infection results in response to different immunization protocols. Recently, Qin et al. (1996) reported that *E. tenella* infection in chickens resulted in a significant increase of *Salmonella enteritidis* in cloacal swabs and eggshells. In poultry, coccidiosis is generally controlled by the prophylactic addition of anticoccidial drugs to the feed. However, the increasing development of the drug-resistant coccidia species and the equally important problem of drug-residues in foods (i.e. meat and eggs) and food safety implications (Donoghue & Hairston, 2000; Kan & Petz, 2000) has stimulated the search for alternative control methods, one of which is represented by vaccination (Chapman, 2000; Ernik & Bedrnik, 2001).

Immunity develops not so quickly after a natural infection but immunization using parasite's extracts or single antigens appears to be more difficult (Vermeulen, 1998). In ground-breeding, one of the most important factor for the developing and diffusion of coccidiosis, which is responsible of poor response to different immunization protocols, is represented by the litter itself, in fact the accumulation of organic material, combined with high levels of temperature and humidity create good conditions for the oocysts sporulation of coccidia. The result of this process is an increase of the protozoan and microbial charge in the litter. At the same time the increase of the ammonia produced per gram of litter by microbial fermentation of faecies produce a bad smell and it is dangerous for chickens, since affects their immunity (Kristensen & Wathes, 2000).

For these reasons with the present trial we wanted to verify the real efficacy of Solvay CRG, a mineral mix used as litter for pets, added to the organic deep litter, as an efficacious method to improve the quality of the litter itself, in association or not with a live attenuated "anti-coccidial" vaccine, comprising all seven *Eimeria* species implicated in the development of disease in poultry, in order to stimulate immunity in elderly pullets, to control coccidiosis and, consequently, to improve animal health and welfare.

MATERIALS AND METHODS

Preliminary trial

A preliminary trial was performed with 20 chicks (Cobb 500) reared on CRG (2-3 cm - deep) from one day to 53 days.

Trial

The research was carried out on 1200 chickens, divided in four groups of 300 animals each, according to the following scheme:

- A1 group: wood shavings litter without CRG - Vaccinated Chicks;
- A2 group: wood shavings litter without CRG - No Vaccinated Chicks;
- B1 group: wood shavings litter with CRG - Vaccinated Chicks;
- B2 group: wood shavings litter with CRG - No Vaccinated Chicks;

CRG Solvay S.p.A. (300-350 g/m^2) was added to the wood shavings litter after the arrival of chicks and again at 35 days (200-150 g/m^2). CRG physiochemical traits are the following: $CaSO_4$ = 65.1%; $Mg(OH)_2$ = 15.4%, $CaCO_3$ = 14.3%, NaCl = 4%, H_2O = 0.9%, SiO_2 = 0.2%, $Ca(OH)_2$ = 0.1%; pH = 10, Specific weight = 0.88, H_2O-Absorbency = 51%; ø = 14% smaller than 0.5 mm, ø = 86% between 6.0 and 0.5 mm. One day old chicks of a colored low growth rate strain fitted for rural production (Plymouth rock type) were used for the trial. The live attenuated vaccine for the seven *Eimeria* species that parasite the domestic fowl (PARACOX(Schering-Plough S.p.A.), was given to the "vaccinated groups", via drinking water, after the 2nd day of rearing.

The animals were reared in 4 separated environments in the same poultry house. Fed was given *ad libitum* without any anticoccidial drug and the same standard of management was carried out in each environment. Clinical controls were performed daily and individual weights (on a sample of about 50 chicks per group) were performed on 25, 33, 40 and 46 days old chicks. At the same ages, a sample of five chicks per thesis was submitted to blood withdrawn then slaughtered for histolopatological, parasitological and bacteriological controls.

Contemporaneously, a sample of litter (10 cm diameter carrot) was collected in each environment near the feeder; pH, ammonia content, bacterial and oocysts count were analyzed. pH was analyzed by HI 8418 Hanna printing pH meter. Ammonia developed by the collected samples was measured after 10 minutes of permanence of litter into a sealed two liter box (1 liter litter and 1 liter air) at ambient temperature (20°C) by a Dräger Rörcher Gas detector (ammonia phial sensibility 5-100 ppm or 100-200 ppm). Dry matter was measured on the same sample used for ammonia determination (static stove at 65°C for 48 hours). The count of the oocysts was measured on McMaster glasses, after suspending 2 g of exhausted litter in 58 cc of an ipersaturated zinc sulfate solution. Specie determination and sporulation times were determined, by microscopic observations on the suspensions obtained by 3 g of exhausted litter and 5 ml of potassium dichromate, at one hour intervals. Suspensions, recovered into Petri capsules, were incubated for 12 hours in the dark at 25°C and sporulation percentages were represented by the number of sporulated oocysts on the total.

Microbial charge was valued according to standard methods: 30 litter grams in 120 ml of peptonated sterile solution (ratio 1:5) accurately mixed and incubated in thermostat at 37°C for 30'; 1 ml of each dilution, 10^{-1} through 10^{-10}, was sown on Standard Plates Count Agar (SPCA, Oxoid) and the colonies were counted after 3 days of incubation at 37°C; the bacterial charge per gram of litter (CFU/g) was obtained by multiplying the average number of colonies of two sow plates (included between thirty and three hundred) for the logarithm of the corresponding dilution.

Metabolic profiles (glucose, cholesterol, triglicerides, total protein, uric acid, albumine, globulins, Ca, P, Mg, Na, K) were analyzed before slaughter on 2 ml of plasma samples obtained by 5 chicks per thesis at each age according to standard methods (Bagliacca et al., 1995). The intestinal parasitary charge was measured on 2 g of the intestinal content of each chick with the same technique described for the litter.

Lesion score, according to Johnson and Reid (1970), and Histological and Parasitological scores, were evaluated on intestine, linphoid organs, liver, and kidneys, by giving a progressive dotting. The histological evaluation of the organ damages and the different stadiums of the biological cycle of Eimeria spp. in the intestinal mucous membrane were evaluated on tissue samples (about 1 cm^3), fixed in 10% buffered formalin. The samples were previously embebbed in paraffin wax then were cut (thickness: 3 μ), mounted on slides and colored with Ematoxylin/Eosin. All dotting started from 0 (no damage) to 4 (heavy damages or high parasitary charge).

Continuous data were analyzed by two way analysis of variance, dotting data were analyzed by non parametric statistics, percentages were analyzed by Chi square (SAS Institute, 1995).

RESULTS AND DISCUSSION

Preliminary trial

The chicks reared on CRG growth regularly, did not show any behavioral modifications and, at slaughter, they did not show any skin lesion or gut alteration, due to the contact or occasionally ingestion of CRG.

Tab. I. Chicken live weights.

Age days		A1	A2	B1	B2	A	B	1	2
		CRG NO		CRG YES					
		Vaccine YES	Vaccine NO	Vaccine YES	Vaccine NO	CRG NO	CRG YES	Vaccine YES	Vaccine NO
	n	47	66	60	74	113	134	107	140
25	**avg**	**237 a**	**275 b**	**241 a**	**279 b**	**259**	**262**	**239 a**	**277 b**
	d.s.	40.7	39.2	34.1	31.9	43.9	37.9	37.0	35.5
	n	40	40	40	40	80	80	80	80
33	**avg**	**533 a**	**594 b**	**630 c**	**715 d**	**563 a**	**673 b**	**581 a**	**654 b**
	d.s.	78.5	77.6	62.2	70.0	79.3	76.3	83.7	94.2
	n	54	54	54	53	108	107	108	107
40	**avg**	**664 a**	**769 b**	**746 b**	**770 b**	**716 a**	**758 b**	**705 a**	**769 b**
	d.s.	134.6	154.8	108.2	132.6	153.6	120.9	128.3	143.5
	n	42	55	53	54	97	106	95	109
46	avg	898	909	871	948	905	911	883 a	929 b
	d.s.	169.8	180.4	149.4	131.5	175.1	145.6	158.4	158.6

Note: means with different letters differ per $p < 0.05$.

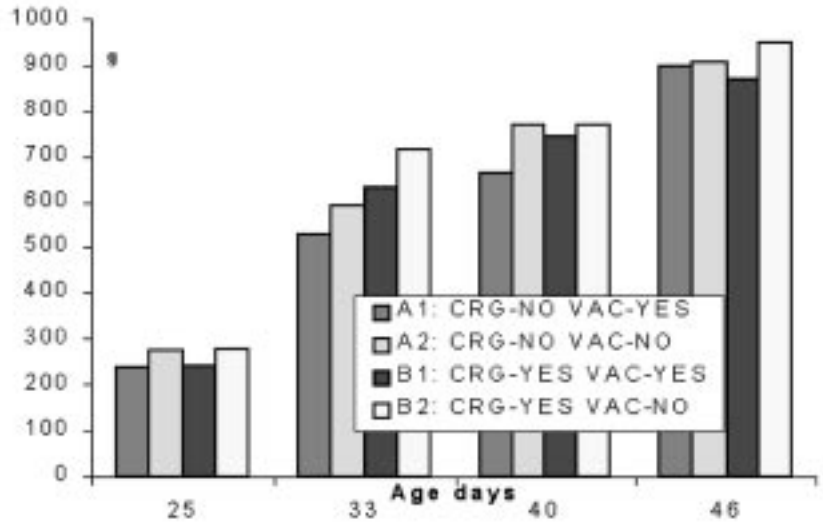

Fig. 1. Chicken live weights.

Trial

Chicken Weight gain (Tab. I, Fig. 1)

The weight gain of the chickens doesn't result reduced by the presence of the CRG in the litter. To the contrary, a tendency to better weight gains can be observed at 33 and 40 days ($p < 0.05$). The performance "shading" observed in the chickens with CRG in the litter at 46 days is probably due to the general worsening of the litter. The weight gain of the chickens doesn't result improved by the use of vaccination. To the contrary, a tendency to worse weight gains can be observed to every age ($p < 0.05$). However It is remarkable the fact that the average weight of chickens growing on litter with CRG is higher than the groups of immunized chickens without CRG, in spite of they were in the period of maximum receptivity to coccidiosis.

Chicken Parasitic charge (Tab. II, Fig. 2)

The McMaster count showed a progressive increase of the oocystic charge in the four groups between the first and the second control, a steady tendency until the third one and then a progressive decrease in the next. Looking at the graph's trend describing the oocystis charge in the four experimental thesis it's interesting to see that we

Tab. II. Oocysts counts.

Age days			A1	A2	B1	B2	A	B	1	2
				CRG NO		CRG YES				
			Vaccine YES	Vaccine NO	Vaccine YES	Vaccine NO	CRG NO	CRG YES	Vaccine YES	Vaccine NO
	25		47	66	60	74	113	134	107	140
oocystis/	33	n/g	9.74 E+02	1.10 E+03	0	3.75 E+03	1.04 E+02	3.75 E+02	4.87 E+02	2.43 E+02
litter	40		1.97 E+04	4.15 E+04	5.21 E+04	1.49 E+04	3.06 E+04	2.68 E+04	3.58 E+04	2.15 E+
	46		2.05 E+04	4.67 E+04	5.73 E+04	5.02 E+03	3.36 E+04	3.12 E+04	3.89 E+04	2.59 E+04
	25		5.12 E+04	4.61 E+04	2.76 E+04	2.54 E+05	4.86 E+04	1.41 E+05	3.94 E+04	1.50 E+05
oocystis /	33	n/g	2.45 E+05	5.63 E+05	3.57 E+05	1.76 E+05	4.04 E+05 b	2.67 E+05 a	3.01 E+05	3.69 E+05
gut	40		1.34 E+06	9.88 E+05	4.78 E+05	2.35 E+05	1.16 E+06 a	3.57 E+05 b	9.07 E+05	6.11 E+05
	46		1.21 E+05	1.45 E+05	3.42 E+05	4.22 E+05	1.33 E+05 a	3.82 E+05 b	2.31 E+05 a	2.84 E+05 b
	25									
oocysts	33	%	83.0	97.0		79.0	90.0 a	79.0 b	83.0	88.0
sporulated/	40		78.0	95.0	58.0	64.0	86.5 a	61.0 b	68.0 a	79.5 b
litter	46		47.0	53.0	28.0	32.0	50.0 a	30.0 b	37.5	42.5
Overall mean			69.3 ab	81.7 a	43.0 b	58.3 ab	75.5 a	56.7 b	62.8 a	70 b
	25									
Sporulation	33	n/h	18.0	20.0		21.0	19.0	21.0	9.0	20.5
time	40		18.0	19.0	20.0	21.0	18.5	20.5	19.0	20.0
	46		19.0	19.0	20.0	20.0	19.0	20.0	19.5	19.5
Overall mean			18.3	19.3	20.0	20.7	18.8	20.5	18.8	20.0

Note: means with different letters differ per $p < 0.05$.

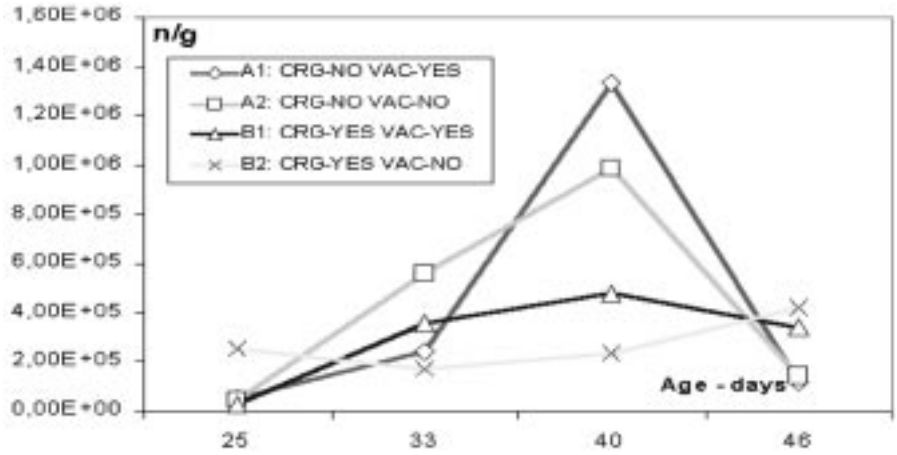

Fig. 2. Oocysts trend in the intestinal content.

found the lowest values in B2 (animals not immunized but with CRG added to the litter) and B1 group (animals immunized with CRG in the litter). The results we got with parasitologic tests on gut's contents of animals slaughtered confirmed the positive action of CRG in the litter, since the parasitic charge was generally lower in subjects bred on litter added with CRG respectively not-immunized and immunized (the coccidia species, isolated and classified after sporulation and measured were: *Eimeria tenella*, *Eimeria maxima*, and *Eimeria mitis*).

Chicken Histopathological evaluations (Tab. III)

The gross and histopathologic examination showed that the incidence of gut's lesions was higher in groups of not immunised chickens even if the parassitic's charge observed was the same. The scores of lesions observed in different organs of the chickens rarely got scores over 2 for the grade of lesions. Considering the influence of CRG on lesion's grade we found in these groups, we can argue that chickens reared on CRG's added litter tend to have lower lesion's scores. A good relationship was found between the macroscopic aspect of intestinal mucosa and the histological one. The absence of micro and macro lesions, in spite of an high grade of infection (Fig. 2), is probably related to the effect of the vaccination of the animals. The oocysts we found had evidently a lower pathogenicity, due to the effects of vaccination.

Tab. III. Histology (lesion scores).

		MACRO				micro			
Gut tract	Age days	A1	A2	B1	B2	A1	A2	B1	B2
Proventricle	25	0	0	0	0	2.5	0.5	1.5	1.5
	33	0.5	0	0.5	0	1.5	1	1.5	1
	40	0.5	0	0.5	0	1.5	0.5	1.5	0.5
	46	0	0	0	0	0.5	0	0.5	0
	53	0	0	0	0	0	0	0	0
Duodenum	25	0	0	0	0	1	0	1	0
	33	0.5	0	0.5	0	1	1	2	0
	40	1	1	2	0	1.5	2.5	2.5	1.5
	46	0	0.5	0.5	0	1	2	1.5	1.5
	53	0	0	0	0	0.5	0.5	1	0
Ileum	25	1.5	0.5	1	1	2	1	2	1
	33	1	0.5	1.5	0	1	1	2	0
	40	1	1	1.5	0.5	1.5	2	2	1.5
	46	0	0	0	0	1.5	2	1.5	2
	53	0	0.5	0.5	0	0.5	1	1.5	0
Caecum	25	2	0.5	2	0.5	2.5	1.5	2.5	1.5
	33	1.5	1	2.5	0	2	2	3	1
	40	3.5	3	3.5	3	3.5	3.5	4	3
	46	3.5	3	3.5	3	3	3	3.5	2.5
	53	1.5	1	2	0.5	1.5	2	2.5	1
Liver	25	0	0	0	0	1.5	1	1.5	1
	33	0	0.5	0.5	0	0.5	1.5	1	1
	40	0	0	0	0	0.5	0.5	1	0
	46	0	0	0	0	1.5	0.5	1	1
	53	0	0	0	0	0	0	0	0

Note: Lesion scores 0 (no damage) though 4 (heavy damages).

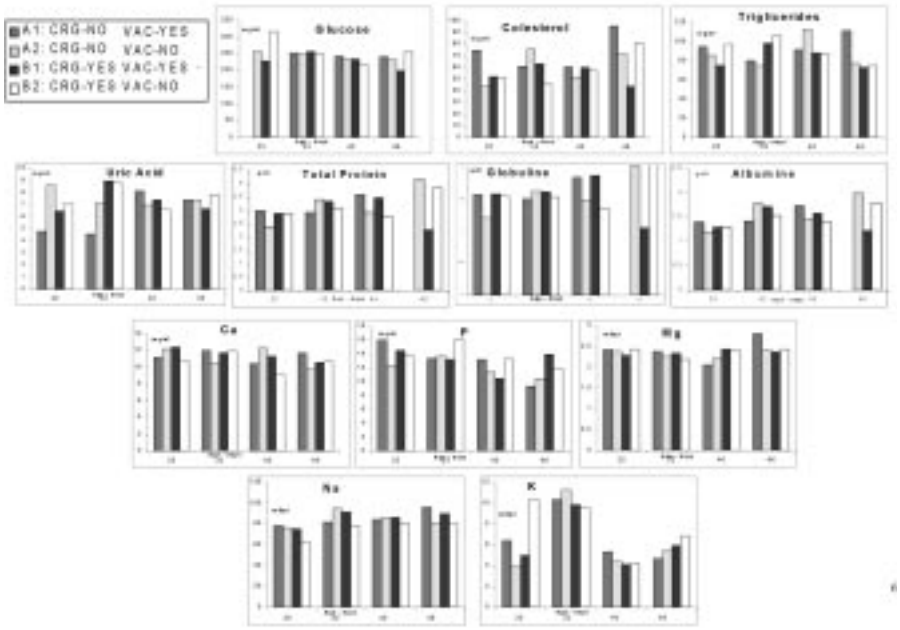

Fig. 3. Chicken Metabolic profiles

Chicken Metabolic profiles (Fig. 3)

Metabolites analysis did not show worsening due to the presence of CRG in the litter. The parameters were very variable and no significant difference were found in relationship to the presence of CRG or the anticoccidial vaccination. *Parameters related to Energy metabolism*. Glucose cholesterol and triglycerid levels, (index of energetic micro deficit, in the field of normality) were compatible in all the chickens with subject in good nutritional state and the observed variations between individuals were not significantly different from the normal distribution. The thesis without vaccination seem however indicate a better absorption (with consequent higher cholesterol levels in the animals reared on the litter with CRG. Such positive trend seems confirmed by the triglicerides, that increase (the last samples) in the thesis with the wood shavings mixed to CRG. *Parameters related to Protein metabolism*. The uric acid (fundamental parameter to allow good weight gains and index of protein excesses, renal problems and/or dehydration at high levels, and spy of protein insufficien-

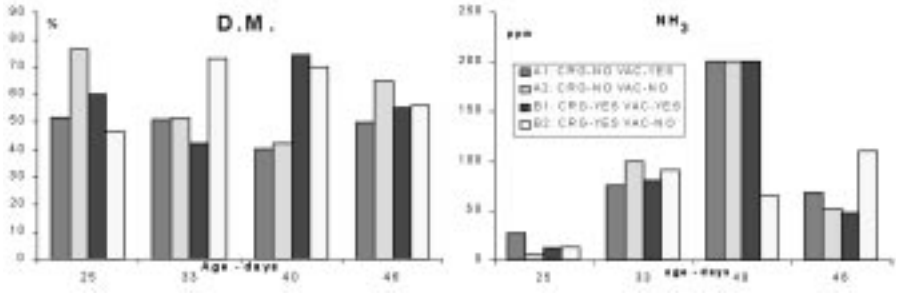

Fig. 4. Dry matter content and ammonia developed by the litter.

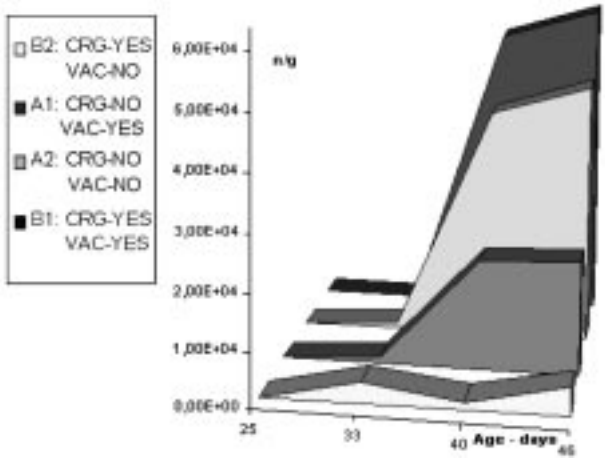

Fig. 5. Oocysts trend in the litter.

cies at low levels), resulted compatible with subject in good nutritional state. The observed variations between chickens were not referable to the experimental thesis. Also the other element of the protein profile resulted in the field of the normality and no variations were observed in relationship to CRG presence or vaccination. *Electrolytes*. a greater presence of an electrolyte can cause a possible deficit in the absorbency/utilize of other electrolytes. In particular K, Na, Ca and Mg are interdependent and the break of the K/Ca balance and an Mg decrease are associated to bad metabolic balances, often due to gastrointestinal troubles. In any case, a trend of plasmatic electrolytes decrease can be observed in correspondence of such troubles (also

Tab. IV. Developed Ammonia (ppm) and water content of the litter (%).

Age days		A1	A2	B1	B2	A	B	1	2
		CRG NO		CRG YES					
		Vaccine YES	Vaccine NO	Vaccine YES	Vaccine NO	CRG NO	CRG YES	Vaccine YES	Vaccine NO
25	NH3	27.0	5.0	12.0	13.0	16.0	12.5	19.5	9.0
	SS	51.5	76.3	60.1	46.3	63.9	53.2	55.8	61.3
33	NH3	75.0	100.0	80.0	90.0	87.5	85.0	77.5	95.0
	SS	50.9	51.2	42.2	72.9	51.1	57.6	46.6	62.1
40	NH3	200.0	200.0	200.0	65.0	200.0	132.5	200.0	132.5
	SS	40.4	42.2	74.5	70.1	41.3 a	72.3 b	57.4	56.1
46	NH3	68.0	52.0	48.0	110.0	60.0	79.0	58.0	81.0
	SS	83.8	83.7	82.5	80.9	83.7	81.7	83.1	82.3
53	NH3	33.0	200.0	200.0	90.0	116.5	145.0	116.5	145.0
	SS	49.7	65.1	55.4	56.0	57.4	55.7	52.5	60.5

Note: means with different letters differ per $p < 0.05$.

light). However, a kalium lowering linked to a sodium saving (increase) was not observed in any sample (as a possible consequence of a starting water retention), so that no metabolic alterations or unbalanced absorption, indirectly or directly related to pathologies (massive infestions can cause electrolytic imbalances), were observed.

Litter Humidity (Tab. IV, Fig. 4)

The water content of the litter, always negative element for poultry rearing seems to decrease in the thesis with CRG added to the litter. the effect results macroscopic in 40 days old chicks (3-4 days after the second spreading of CRG on the surface of the litter). The general opinion of the poultry breeders of the improvement of the litter which is obtained with the addition of CRG, may be explained, at least partially, by such effect. However it is not possible to operate continue and constant CRG additions to the litter when the litter seem to be exhausted, on account of cost reasons. In fact both CRG or a wood shavings addition, can give further improvement to the litter without causing alterations to the chickens.

Litter Ammonia production (Tab. IV, Fig. 4)

The average ammonia production in the four litter was very high from a welfare point of view (maximum values of 200 ppm were reached!). However, ammonia production was very variable and no significative difference was observed between thesis (only a light tendency to lower values was observed with CRG at 25,33 and 40 days).

Litter Parasitic charge (Tab. II, Fig. 5)

The McMaster count showed a progressive increase of the oocystic charge in the four groups between the first (33 days) and the second (40 days) control and a steady tendency until the third one (46 days). Looking at the graph's trend describing the oocystis charge in the four experimental thesis it's interesting to see that we found the lowest values in B2 (animals not immunized but with CRG added to the litter) and A1 groups (animals immunized without CRG in the litter).

Litter Oocysts Sporulation: time and percentage (Tab. II)

Concerning of parasitic charge we found the highest values of

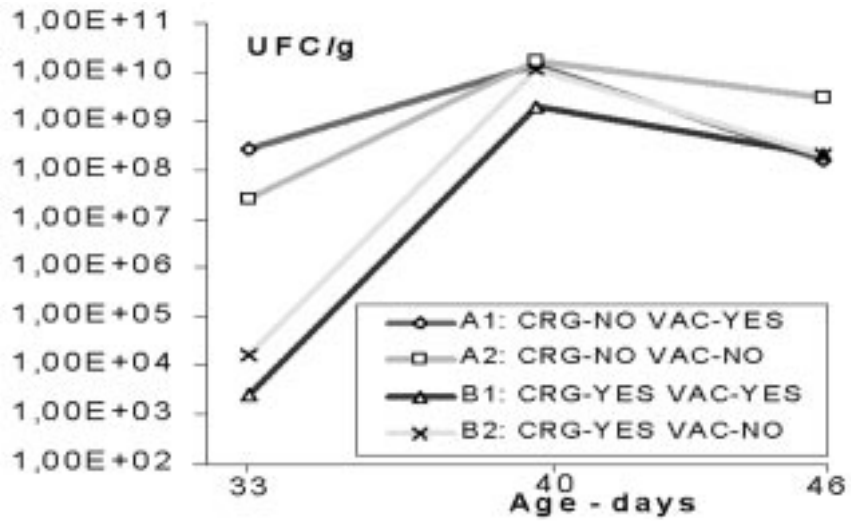

Fig. 6. Bacterial counts (Units Forming Colonies) in the litter.

sporulation in litters not added with CRG. We can desume on the bases of our results that the addition of this salt to the litter may drastically reduce the sporulation rate of the oocysts. In fact the overall mean of sporulated oocysts was 75.5% without CRG and decreased to 56.7% with CRG in the litter, where a large number of abortifacent oocysts could be found.

Sporulation times were very high but the identification of the different species can get a correct evaluation of the alterations of the sporulations' times and their lack (*Eimeria tenella*, *Eimeria maxima*, and Eimeria mitis were isolated and classified after sporulation).

Litter Bacterial charge (Fig. 6)

Bacterial charge (CFU per g of litter) was systematically lower in litters added with CRG. This fact, correlated with decreasing trend of the water levels (Fig. 2), improves the quality of the litter. The group A2 (not immunized and without CRG) showed the highest microbial charge per gram of litter and consequently developed the worst environment for the chickens.

CONCLUSIONS

Results showed that CRG presence in the deep litter house does not reduce the chicken weight gain but the live weight trends improve (in 33 and 40 days old chicken, CRGno = 563 g and 716 g; CRGyes = 673 g and 758 g). The presence of CRG improves also the dry matter of the litter (40 days, CRGno = 41.3% and CRGyes = 72.3%). The lower humidity rate of the litter, associated with the higher saline concentration, related to CRG presence, probably cause a reduction of "Free" or Active Water (AW). The reduction of AW may explain the decrease of the protozoa counts in litter and oocysts sporulation (mean observed during the trial, CRGno = 75.5%, CRGyes = 56.7%). In addition, the reduction of AW can be involved into the improvement of the bacteriological charge during the trial.

Ammonia developed by litter and metabolic (energy and protein) profiles of chickens, were very variable during the trial and no significant differences between thesis were observed. Finally, the fact that the most significant lesions and histological scores were observed in the group of unvaccinated birds that were maintained on common litter (without CRG addition), demonstrates the importance of immunization and litter hygiene in preventing the disease.

Regarding the litter itself, CRG added litters may have good employing for fertilization of alkaline grounds. The positive effects of poultry manure on crop yields is well known (Lysenko, 2001) but the presence of CRG may improve the quality of the manure itself. In fact the combination between wet litter and animal's faecies produces ammonia sulfate which is a physiologically acid manure, helpful to correct alkaline grounds: the ammonia is adsorbed and employed by plants while the sulforic group remains in the ground. The ammonia sulfate, widely soluble in water, is formed between calcium sulfate of CRG and ammonia and carbon dioxide developed by the microorganism of the litter: $CaSO_4 + 2NH_3 + CO_2 + H_2O \rightarrow CaCO_3 + (NH_4)2SO_4$. In addition, also the possible remaining calcium sulfate is useful to correct the excessive alkalinity of the ground: alkalinity linked to the presence of salts in the circulating solutions or cations absorbed by the colloids of the ground. The action mechanism is linked to the reaction between soluble sodic carbonate (reliable of alkalinity) and the sodium sulfate contained in the CRG that forms insoluble calcium car-

bonate and neutral sodic sulfate: $CaSO_4 + Na_2CO_3 \rightarrow CaCO_3 + Na_2SO_4$.

In conclusion results suggest that the use of CRG in deep litter houses associated with a live attenuated anticoccidial vaccination may be an efficacious method to control coccidiosis and, consequently, an alternative way (to anticoccidial drugs in feed) to control the disease in poultry production. Finally CRG improve the quality of the litter (which must be used for alkaline or saline grounds).

REFERENCES

ABRAHAMSSON P., FOSSUM O., TAUSON R. (1998). Health of laying hens in an aviary system over five batches of birds. Acta Vet. Scand., 39 (3): 367-379.

BAGLIACCA M., PACI G., MARZONI M., SANTILLI F., BIAGI G. (1995). Effetto del diverso contenuto di fibra del mangime sullo sviluppo intestinale e sul profilo metabolico dei fagiani in accrescimento (Proc. of the VI Congress of International Society for Animal Clinical Biochemistry, Guelf, Canada 1994: 105). Riv. di Avicoltura, 65 (1-2): 33-39.

BAFUNDO K.W., DONOVAN, D.J. (1988). Predicting effects of coccidial lesions in broilers. Poultry, 4 (4): 36-37.

CHAPMAN H.D. (2000). Practical use of vaccines for the control of coccidiosis in the chicken. World's Poultry Science Journal, 56 (1): 7-20.

DONOGHUE D.J, HAIRSTON H. (2000). Food safety implication: certain antibiotics may rapidly contaminate egg albumen during the process of its formation. British Poultry Sci., 41 (2): 174-177.

ERNIK F., BEDRNIK P. (2001). Controlling coccidiosis in broiler growing. Poultry International, 40 (4): 36-42.

HEIJMANS J.F., BRAUNIUS W.W., BEERSMA P.F., DE WITT J.J. (1990). A case of cryptosporidiosis in breeding laying hens with Marek's disease. Tijdschr Diergeneeskd, 15; 115 (14): 673-675.

JOHNSON J., REID W.M. (1970). Anticoccidial drugs: Lesion scoring techniques in battery and floor-pen experiments with chickens. Experimental Parasitology, 28: 30-36.

KAN C.S., PETZ M. (2000). Residues of veterinary drugs in eggs and their distribution between yolk and white. J. Agriculture Food. Chem., 48 (12): 63-97.

KRISTENSEN H.H., WATHES C.M. (2000). Ammonia and poultry welfare: a review. World's Poultry Science Journal, 56 (3): 235-246.

LYSENKO V.P. (2001). Poutry manure is a valuable fertilizer. Poultry International, 40 (11): 54-56.

QIN Z., ARAKAWA A., BABA E., FUKATA T., SASAI K. (1996). Effect of Eimeria tenella infection on the production of Salmonella enteritidis-contaminated eggs and susceptibility of layng hens to S. enteritidis infection. Avian Diseases, 40 (2): 361-367.

SAS INSTITUTE (1995). JMP, SAS Institute Inc., Cary NC.

TROUT J.M., LILLEHOJ H.S. (1996). T lymphocyte roles during Eimeria acervulina and Eimeria tenella infections. Vet. Immunol. Immunopathol., 53 (1-2): 163-172.

VERMEULEN A.N. (1998). Progress in recombinant vaccine development against coccidiosis. a review and prospects into the next millennium. Int. J. Parasitol., 28 (7): 1121-1130.

WILLIAMS R.B., CARLYLE W.W., BOND D.R., BROWN I.A. (1999). The efficacy and economic benefit of Paracox, a live attenuated anticoccidial vaccine, in commercial trials with standard broiler chickens in the United Kingdom. Int. J. Parasitol., 29 (2): 341-355.