A novel approach to coccidiosis control

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CoxAbic is a new concept in coccidiosis control. The concept is based on vaccinating breeders with a sub-unit vaccine produced from a protein extracted from the gametocyte stage of E. maxima. The sub-unit protein complex is called APGA (Affinity Purified Gametocyte Antigens) and is composed of an 82 kDa protein and a 56 kDa protein. Vaccination with APGA induces production of antibodies that specifically react with the Wall Forming Bodies (WFBs) in the gametes. The WFBs eventually form the hard protective wall of the oocyst. The specific APGA proteins appear to be well conserved among the common broiler chicken species of Eimeria (E. acervulina, tenella and maxima).

The breeder birds are vaccinated twice with CoxAbic, four weeks apart. The vaccine hyperimmunises the breeders, producing specific antibodies that are passed to their broiler chicks through the egg yolk. These antibodies are thought to interfere with the wall formation of the oocysts. This specific maternal immunity protects the chicks during the initial exposure to coccidia in the litter, permitting the development of active immunity against the species of Eimeria found in the litter. The level of immunity can be measured in the vaccinated breeders and in the broiler offspring by using a specific ELISA test. In this manner the results of vaccination can be verified.

In large production systems where the vaccine is currently being used, performance of broiler chicks in the field is better than those grown with the use of coccidiostats or live vaccines. In addition, improved quality of life of the vaccinated breeders has been repeatedly observed when compared to non-vaccinated controls. Egg production is also increased in vaccinated breeders.

Coccidiosis, an infection caused by parasitic protozoa of the genus, Eimeria, continues to be the most reported disease in broiler production around the world, causing significant economic damage to poultry operations.

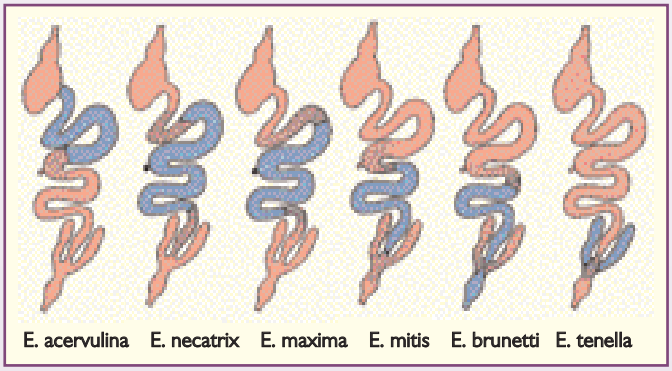
The sub-clinical form is frequently the subject of misdiagnosis as it is often confused with other causes of enteritis.

Coccidiasis – that is, infection without significant pathology or economic impact – occurs on all poultry farms, demonstrating the total impossibility of eradicating this agent.

Coccidiosis control in chickens is primarily based on the addition of anticoccidial products to feed rations, as introduced in the 1970s.

This method is constantly undergoing restrictions for the purpose of eliminating any residue from carcases that could potentially harm, or be perceived by the consumer to harm human health.

Fig. 2. Identification of coccidial species according to the location of the lesions.



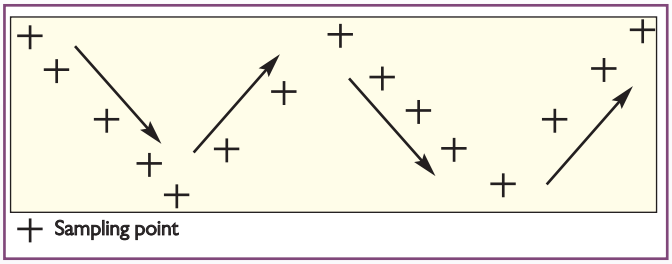


Fig. 1. Approximate position of sampling points (for house with 10,000 broilers).

The first viable alternative to anticoccidials was the use of live vaccines, attenuated or virulent in the field, with a precocious cycle or not. Although already established commercially, the use of vaccines encounters resistance from producers.

This is primarily due to the high cost, the need to handle the birds, the likelihood of some degree of injury, and the need to use Eimeria strains that are prevalent in the region because of species specific or even strain specific immunity.

Molecular basis of action

CoxAbic provides protection against coccidiosis in a unique way.

Breeders injected with CoxAbic produce large amounts of antibodies against the proteins contained in the vaccine, most especially an 82 kDa protein – gam82 – and a 56 kDa protein – gam56 – purified from the gametocytes of Eimeria maxima.

These antibodies are transferred from the bloodstream of the breeders into the yolks of developing eggs.

As the embryonic chicks mature, they absorb the antibodies contained within the yolk into their own bloodstream, particularly in the last few days before hatching and the first few days after hatching from the egg. It is these maternal antibodies that protect the chicks against infection with Eimeria.

Gam56 and gam82 play a crucial role in the development of Eimeria parasites. Both these proteins are found in the WFBs of the macrogametes of E. maxima.

These WFBs – put simply – fuse together to form the oocyst wall, a hard, impermeable structure that protects the parasites as it is excreted in the faeces of chickens and also protects them from various chemicals and disinfectants in the outside world.

The oocyst wall ensures that Eimeria is efficiently transmitted from chicken to chicken.

The secret to this success is the fact that gam56 and gam82 are both rich in a particular amino acid, tyrosine, which is capable of forming strong chemical binds that unite two tyrosine molecules (referred to as dityrosine bonds) between proteins.

This leads to dehydration and hardening – a process known as 'tanning' – to produce the distinctive oocyst wall.

Vaccination with CoxAbic causes the production of antibodies that somehow prevent the formation of dityrosine bonds and their associated protein matrices, thereby inhibiting formation of the oocyst wall and, therefore, transmission of Eimeria parasites between chickens.

The effect is seen not just against E. maxima – the species from which gam56 and gam82 are purified – but also against other Eimeria species (for example, Eimeria tenella and Eimeria acervulina) because of the apparent conservation of these proteins in various species of Eimeria.

There are several advantages in using CoxAbic – resistance cannot be established because the vaccine does not exclude the parasite but allows it to infect the chicks in a controlled and mild way; no withdrawal

Continued on page 8

Continued from page 7
period is required because there are no residues and there is no risk of damage to performance by over- and under dosing and there is no interference with routine production practices as there is no risk of giving the wrong (medicated) ration to a flock, since no anticoccidials are used and no additional medications are required.

This technology is already commonly used in Israel and South Africa.

Field efficacy tests began in Brazil in 2003 and included three phases:

- Phase 1 – Breeder safety phase.
- Phase 2 – Broiler efficacy phase.
- Phase 3 – Collation of data record sheets by trial supervisor, completion of laboratory tests, statistical analysis of data, and preparation of final study report.

Litter sampling

Starting at two weeks after placement, litter was collected weekly from test and control broiler houses by the broiler farm supervisor.

Litter sampling was performed in duplicate, with paired samples being collected from each sampling point and placed into separate collection bags.

The samples were pooled within each duplicate so that at the end of the sampling procedure two collection bags containing pooled samples representative of the whole house were obtained.

The samples were collected by taking a large pinch of litter from the ground, with care being taken to ensure the samples were of a similar size.

Fifteen points were sampled. Samples were taken along a roughly W-shaped path starting and finishing in the corners of one of the long sides of the house as indicated in Fig. 1.

Once collection was finished, completed copies of identification papers were placed inside the collection bags and the bags closed using plastic ties.

	CoxAbic chicks	Medicated control chicks	Difference
Mortality (%)	5.88	5.84	0.04
Daily weight gain (g)	57.4	54.7	2.7
Feed conversion	2.09	2.18	0.09
Weight (kg)	2.810	2.680	0.130
Index	259	237	22
Age at slaughter (days)	49	49	-

Table 2. Trial No. 1. Broiler performance, raised on 10th March 2003. 15,000 CoxAbic chicks versus 15,000 chicks fed with anticoccidials.

Table 3. Trial No. 2. Broiler performance, raised on 9th June 2004. 23,200 CoxAbic chicks versus 23,200 chicks feed with anticoccidials.

	CoxAbic chicks	Medicated control chicks	Difference
Mortality (%)	3.84	3.00	0.84
Daily weight gain (g)	57.9	59.3	1.4
Feed conversion	1.77	1.81	0.04
Weight (kg)	2.720	2.790	70
Index	311	315	4
Age at slaughter (days)	48	48	-

Each closed collection bag was then placed inside another plastic bag and another completed copy of an identification paper inserted between the collection bag and the outer plastic bag.

Litter samples were kept in a shaded and cool place during storage and transport.

The litter sample bags were placed on ice in insulated boxes and transferred to the laboratory for oocyst counts within 24-36 hours.

Oocyst counting

Oocyst counts were performed within 24-36 hours of receipt of the litter samples at the laboratory.

In total, not more than 72 hours occurred between collection of litter samples and performance of oocyst counts.

At the laboratory, the litter samples were refrigerated at 4°C until they were processed for counting.

The oocyst counting procedure is as follows:

- Mix well the contents of the litter sample bag by shaking it thoroughly.
- Weigh two samples of 10g of litter. Run the two samples as duplicates and record both counts separately.
- To each sample add 100ml of water and store in the refrigerator (4°C) overnight.

- Mix the suspension well for one minute with a low speed electric mixer.
- Filter the suspension through a metal sieve. Press out remaining drops using a pestle.
- Take a 10ml sample of the filtered solution into a test tube and centrifuge for five minutes at 1500 rpm.
- Pour off the supernatant. Suspend the pellet precipitate in a saturated solution of NaCl in water and bring the volume back to 10ml.
- Mix well and place a sample of the suspension into a McMaster counting chamber. Count the floating oocysts using a microscope.
- Calculate the number of oocysts per gram of litter by multiplying the counted oocysts by 67.

Lesion scoring

Starting at week two, five randomly selected broilers from each group were euthanised at weekly intervals and subjected to necropsy by a qualified person.

Any coccidial lesions found in the intestine were scored.

The examination took place within 15 minutes of euthanasia in order to permit identification of E. acervulina lesions.

The scoring was performed by the same qualified person throughout the trial and was performed according to the following procedure:

- Dissect out the digestive tract and examine the unopened intestines.
- Perform a longitudinal incision along the intestine to permit the examination of the mucosal wall and the intestinal contents.
- Make a preliminary identification of coccidial species causing the lesions according to the location of the lesions as illustrated in Fig. 2.

Where a clear distinction between two species could not be made based on location (for example, between E. maxima, E. necatrix and/or E. mitis), the scoring tables below were used as an aid for differential diagnosis:

- Obtain mucosal scrapings from areas where lesions are found for microscopic analysis to confirm the presence of coccidia.

In the absence of gross lesions, scrapings should be taken from the following sites to confirm the presence or absence of coccidia – duodenum, mid-small intestine, around the yolk sac, diverticulum, caeca, and between the tips of the caeca and the cloaca.

- To score the lesions, the following aspects were considered – location (E. acervulina, E. mitis and E. brunetti only), gross appearance, number, condition of the intestinal wall and the intestinal contents. Scores were assigned as described (see inset right) for each species.

A score was assigned to each category and a mean was calculated of

Continued on page 11

Lesions	Scoring 1	Scoring 2	Scoring 3	Scoring 4
E. ACERVULINA				
Location	duodenum	up to 20cm beyond duodenum	up to yolk-sac diverticulum	up to yolk-sac diverticulum
Gross appearance	long white patches, ladder-like aspect	long white patches, ladder-like aspect but more coalescent	Lesions coalesced to give coated appearance	grayish mucosa with coalescent colonies
Number of lesions	max 5/cm^2	very numerous	coalescent lesions	completely coalescent
Condition of intestinal wall	normal	normal	thickened	greatly thickened
Intestinal contents	normal	normal	watery, slimy	creamy exudate
E. NECATRIX				
				(dead birds)
Gross appearance	scattered petechiae, white patches on serosa	numerous petechiae on serosa, violent red	extensive red petechiae, and white plaques on serosa	dark colour, extensive haemorrhages
Number of lesions	scattered	numerous	numerous	coalescent
Condition of Intestinal wall	normal	swelling limited to central part	rough, thickened and swollen up to lower part	swelling over whole intestine
Intestinal contents	normal	normal	extensive haemorrhage red or brown mucus	very dark red or brown mucus
E. MAXIMA				
				(dead birds)
Gross appearance	small, red petechiae, bleeding	red speckled petechiae	mucosa surface rough and bloody red	bright red colour, putrid smell
Number of lesions	scattered	numerous	coalescent	coalescent
Condition of intestinal wall	normal	thickened	thickened and swollen	greatly thickened and swollen
Intestinal contents	sometimes orange mucus	orange mucus	mucus, pinpoint blood clots	many blood clots and digested red blood cells
E. MITIS (MIVATI)				
Location	duodenum	up to 20cm beyond duodenum	up to lower small intestine	the entire small intestine
Gross appearance	round white patches, red coloured	round white patches, red coloured but more coalescent	round white patches, red coloured but more coalescent	round white patches, red coloured but more coalescent
Number of lesions	max 5/cm^2	very numerous	no coalescence	no coalescence
Condition of intestinal wall	normal	normal	thickened	greatly thickened
Intestinal contents	normal	normal	watery, slimy	creamy exudate
E. BRUNETTI				
Location	lower small intestine	lower small intestine	lower small intestine, rectum, caecum	small intestine (extending to middle and upper), rectum, caecum
Gross appearance	no severe lesions some graying or reddening of the mucosal surface	sometimes grey colour, salmon coloured flecks	bloody, transverse red streaks in rectum. Lesions may occur in caecal tonsils	bloody and necrotic lesions
Number of lesions	scattered	scattered	coalescent	coalescent
Condition of intestinal wall	normal	thickened up to lower part	thickened and swollen	thickened, caseous cores may plug the caeca
Intestinal contents	normal	normal	bloody and watery, sometimes soft slime balls	sometimes caseous clots obstruct caecum
E. TENELLA				
Gross appearance	petechiae	petechiae	bloody	bloody, bluish-black
Number of lesions	scattered	more numerous	coalescent	coalescent
Condition of intestinal wall	normal	somewhat thickened	greatly thickened	greatly swollen and thickened
Intestinal contents	normal	normal caecal contents but with some blood	much blood and many fibrin clots or caecal cores, little or no faecal debris	blood, caseous clots, no faecal debris

Continued from page 9

the individual scores, to give a global lesion score.

Results

● **Efficacy test I.**

From the same breeder farm, two breeder houses were vaccinated with CoxAbic, and two remaining breeder houses remained as non-vaccinated controls.

The offspring of both flocks (CoxAbic vaccinated and non-vaccinated) were raised in a commercial broiler farm, under the same management conditions, but in separate broiler houses.

The offspring from the non-vaccinated control breeder flock was fed anticoccidial drugs in the feed, at the usual doses until seven days to slaughter.

	CoxAbic	Live vaccinated control	Difference
Mortality (%)	4.48	5.10	0.62
Daily weight gain (g)	49.5	49.3	0.2
Feed conversion	2.02	2.02	-
Weight (kg)	2.296	2.296	-
Index	235.0	233.3	2
Age at slaughter (days)	46.36	46.50	-

Table 4. Total field performance. Broiler performance, from August 2004 to March 2005, 2,552,342 birds.

The mortality, daily weight gain, feed conversion, weight at slaughter and Index data were collected from the company's normal data spreadsheet.

● **Efficacy test II:**

From the same breeder farm, two breeder houses were vaccinated with CoxAbic, and two remaining breeder houses remained as non-vaccinated controls.

The offspring of both flocks (CoxAbic vaccinated and non-vaccinated) were raised in a commercial broiler farm, under the same man-

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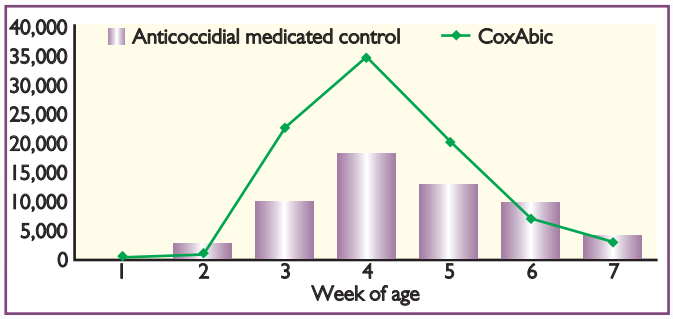


Fig. 3. Efficacy test I. Faecal coccidial oocyst counts, 625,000 broilers involved from 2003 to 2004.

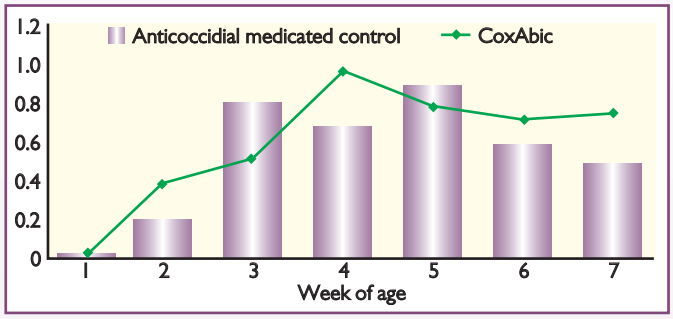


Fig. 4. Efficacy test I. Lesion scores, 625,000 broilers involved 2003 to 2004.

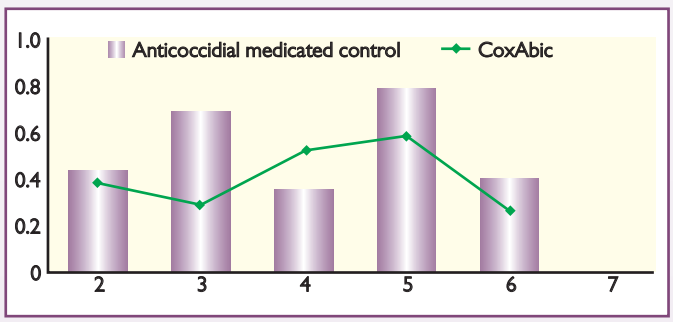
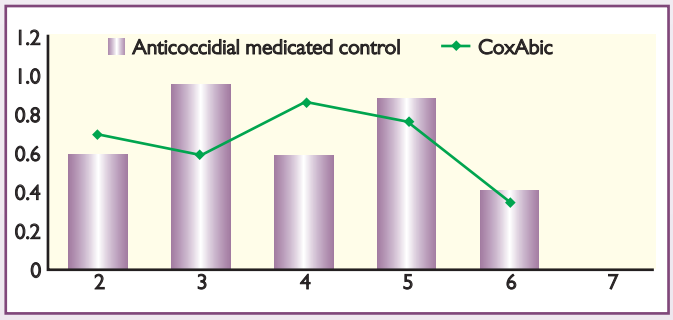


Fig. 5. Eimeria acervulina lesion scores, 625,000 broilers from 2003 to 2004.

Fig. 6. Eimeria maxima lesion scores, 625,000 broilers from 2003 to 2004.



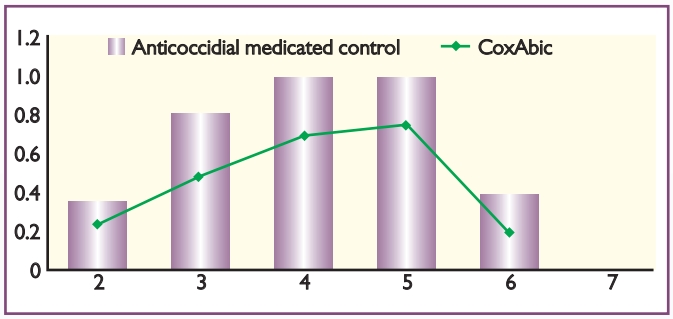


Fig. 7. Eimeria tenella lesion scores, 625,000 broilers from 2003 to 2004.

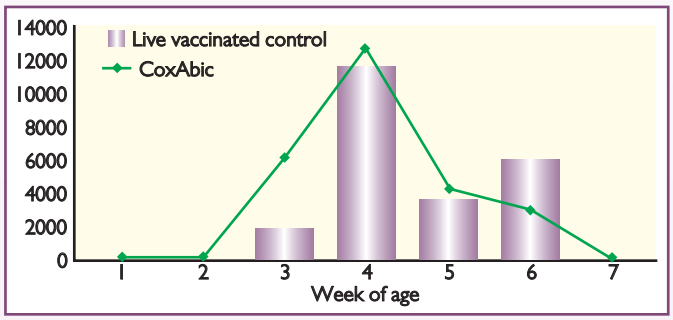


Fig. 8. Efficacy test II. Faecal coccidial oocyst counts 282,800 broilers involved from August 2004 to March 2005.

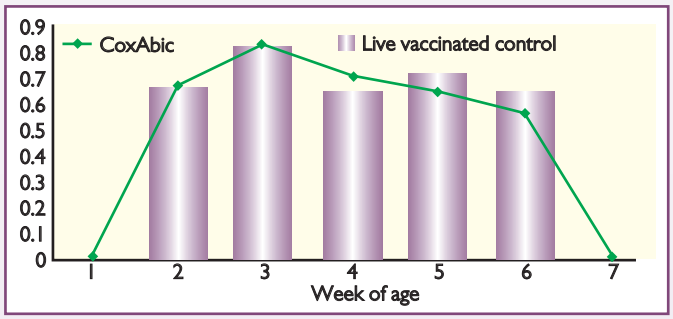
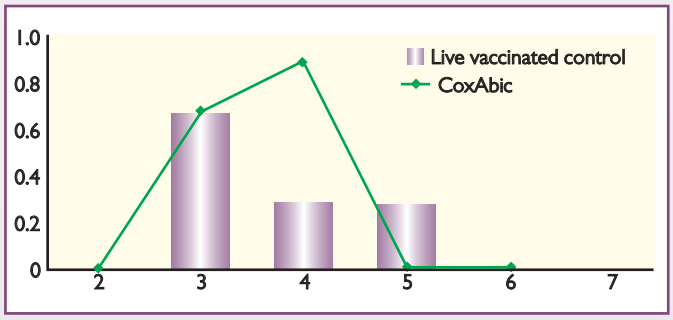


Fig. 9. Efficacy test II. Lesion scores, 282,800 broilers involved August 2004 to March 2005.

Fig. 10. Eimeria acervulina lesion scores, 282,200 broilers, August 2004 to March 2005.



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agement conditions, but in separate broiler houses.

The offspring from the non-vaccinated control breeder flock was vaccinated with live vaccines against coccidiosis at the first day of age as per label recommendation.

The mortality, daily weight gain, feed conversion, weight at slaughter and Index data were collected from the company's normal data spreadsheet.

	Birth date	No. of vaccinated breeders	CoxAbic female livability 66 weeks of age	Control female livability 66 weeks of age	CoxAbic hatchable eggs production/ hen housed	Control hatchable eggs production/ hen housed
Breeder safety test I	15th Dec. 2002	19,455	84.42	82.18	176.52	176.52
Breeder safety test II	17th June 2003	20,218	88.79	85.04	176.43	169.60
Breeder safety test III	20th Dec. 2003	18,734	95.91	95.86	192.01	187.78
Breeder safety test IV	24th Dec. 2003	18,500	90.07	86.86	179.74	178.02
Breeder safety test V*	2nd Feb. 2005	39,652	95.87	NCF	61.25	NCF

*Actual flock age = 37 weeks NCF = No control flocks

Table 5. Breeder safety test results.

No side effects seen

The vaccinated breeders did not show any side effects or adverse reactions to the vaccine.

A preliminary study indicates that the vaccinated breeders laid a few more eggs and had a slightly lower mortality than their unvaccinated sisters. These parameters need further study and confirmation.

During Efficacy test I, oocyst shedding peaked, in the CoxAbic flocks as well as in the control flock, at four weeks of age. Oocyst shedding of CoxAbic chicks was two times higher than that of the chicks fed with anticoccidials, however, lesion scores were similar, showing protection against E. maxima, E. acervulina and E. tenella, in the CoxAbic chicks. This demonstrates that there is a low correlation between the number of coccidial oocyst shed and gut lesion scoring and clinical signs of coccidiosis.

During Efficacy test II, the oocysts shedding also peaked at four weeks of age, with CoxAbic chicks shedding similar amounts of oocyst as chicks given live vaccines.

The lesion scores of both groups were similar, demonstrating the protective effect of both coccidiosis control systems. This reinforces the view that coccidial oocyst numbers and/or degree of gut lesion scores bear little relation to the final performance of the birds.

During all efficacy tests coccidiosis challenge occurred in the field as expected. Some flocks fed anticoccidials and some flocks vaccinated with live vaccine, had clinical signs of coccidiosis and were medicated. None of the CoxAbic flocks needed medication, demonstrating the effectiveness of CoxAbic vaccination.

Conclusion

Offspring from CoxAbic vaccinated breeders showed similar performance to those fed with anticoccidial drugs, during the breeders' normal productive lifespan.

Offspring from CoxAbic vaccinated breeders shows similar performance to those vaccinated with live coccidia vaccines at one day of age, during the breeders' normal productive lifespan.

Production results on the farms with CoxAbic chicks revealed a decrease in mortality, an equivalent FCR and superior weight gain compared with chicks immunised with live vaccines.

This resulted in a two point improvement in European Efficiency Index in flocks derived from CoxAbic vaccinated breeders. ■

Fig. 11. Eimeria maxima lesion scores, 282,200 broilers, August 2004 to March 2005.

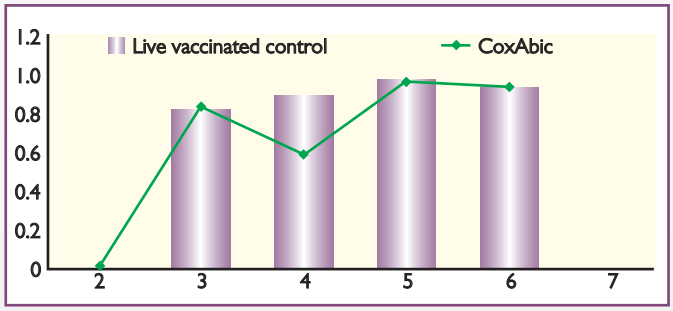

Fig. 12. Eimeria tenella lesion scores, 282,200 broilers, August 2004 to March 2005.