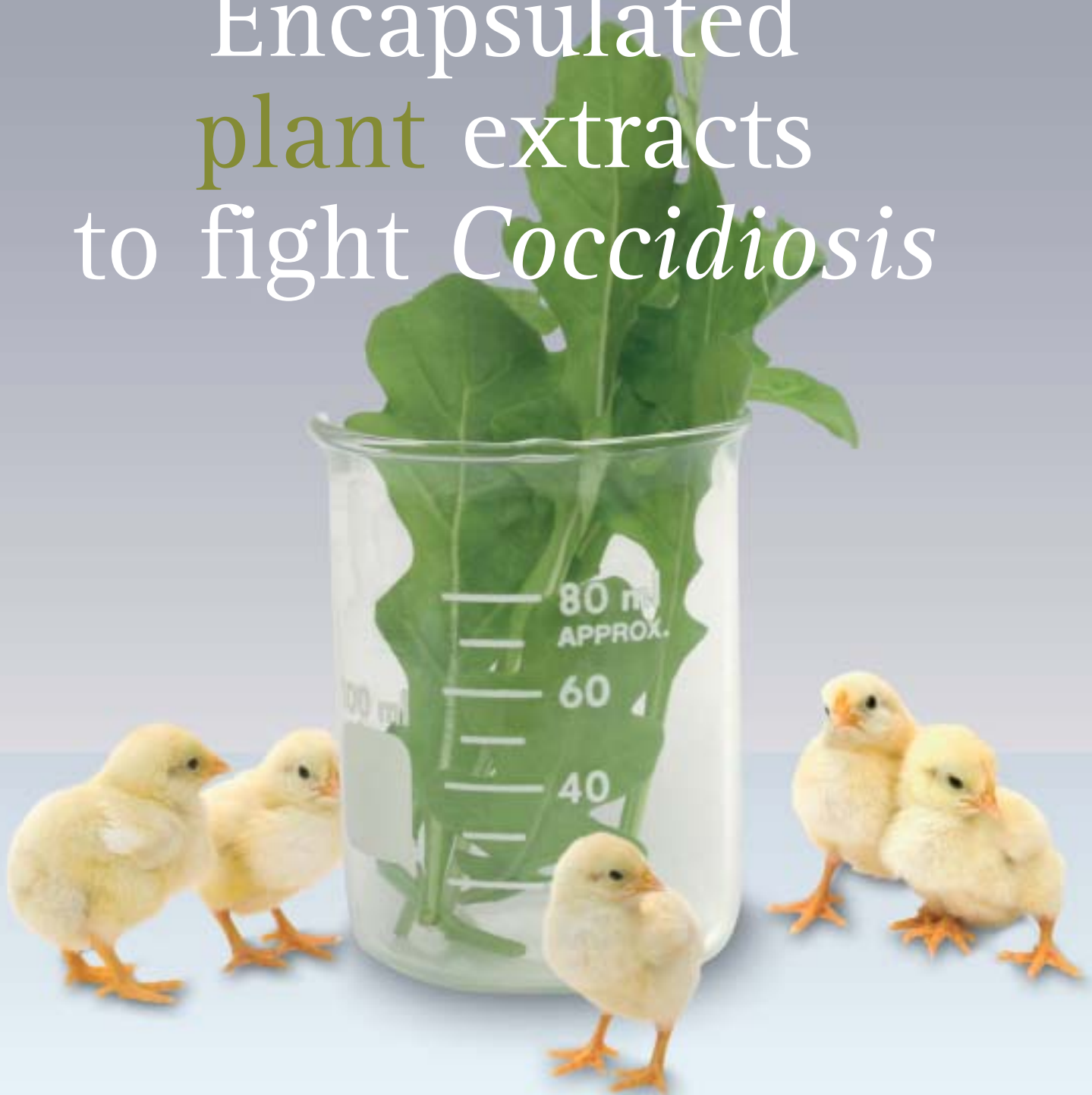


Encapsulated plant extracts to fight *Coccidiosis*



A THREE-YEAR PHD STUDY AT THE UNIVERSITY OF LEEDS AND CONTINUING RESEARCH HAVE SHOWN THE POTENTIAL FOR THE COMBINATION OF THYMOL AND CARVACROL AS A FEED MICROADDITIVE TO LIMIT THE PRODUCTION IMPACT OF EIMERIA CHALLENGE. HENRY GREATHEAD AND CHRIS KAMEL EXPLAIN.

Coccidiosis is a disease caused by protozoan parasites of the genus *Eimeria* that can seriously affect the health and productivity of livestock, particularly in modern, intensive production systems. It results in a decline in general health, a failing appetite and eventually a loss of condition, and under severe conditions, even death. Infected livestock are also more susceptible to other diseases. From an economic perspective, coccidiosis is considered one of the top three costly pathologies in young animals with annual global economic losses estimated at over €1-1.5 billion each in the broiler and cattle areas alone (USDA Agricultural Research Service Newsletter, 2005). To prevent livestock suffering and to limit economic losses, anticoccidial drugs (coccidiostats) have been and continue to be routinely and successfully used. However, the emergence of drug-resistant strains of *Eimeria*, consumer concerns over in-feed prophylactic drug inclusion, and the anticipated withdrawal of coccidiostats at the end of 2012 means alternative methods of controlling coccidiosis need to be considered. Vaccines are being developed and alternative feed additives based on bioactive compounds found in plants are currently being investigated.

FINISHING NATURE'S CREATION

A three-year independent project conducted at the Faculty of Biological Science at the University of Leeds investigated the use of plant extracts to manage coccidiosis in broiler chickens caused by *Eimeria acervulina*, one of the species that infect poultry. Preliminary feeding experiments highlighted the potential of components of the essential oil from *Origanum spp.*, thymol and carvacrol.

But where to start? Essential oils are complex mixtures of ingredients. Instead of investigating each and every component, the project focused on the two prime ingredients, thymol and carvacrol. However, the relative concentrations of thymol and carvacrol in *Origanum* essential oil varies considerably, being affected by species of *Origanum*, growing environment, the part of the plant extracted and the method of extraction. Results led to the conclusion that the combination of thymol with carvacrol at a 1:1 ratio yielded more promising results on performance and intestinal characteristics than either ingredient alone. It is proposed that this may be due in part to the stability of these ingredients. This was supported by the work of Jukic and Milos (2004) who showed that both of these ingredients when present alone had the capacity to undergo oxidative transformation into a different molecule. This oxidation was spared only when the two ingredients are paired together due to the inherent anti-oxidation properties of these compounds.

MORE IS NOT BETTER

Defining the optimal dosage is a critical step in the development of a feed additive. However, to date there are very few suppliers of alternative feed additives that have devoted time and expense to provide this information. In order to understand the dose-response of the standardised combination of thymol and carvacrol (NE), several studies were performed to define the optimal level. One of these is shown in *Figure 1*. In this study, broilers challenged with 500,000 sporulated *E. acervulina* oocysts at 23 days of age, were fed a ground commercial diet (86% dry matter, 14% protein, 5% fibre and 5% fat, fresh weight basis) supplemented with a 1:1 combination of thymol:carvacrol (NE) at different dosages from 125-2000 g/t. These were compared to a control group of broilers uninfected and untreated with NE. Broilers fed the lower inclusion levels of NE (IT1, IT2) performed significantly ($P < 0.05$) better than those fed NE at higher levels. At the low levels, NE reversed the anorexic effects of growth on the *Eimeria* challenge, such that by day 11 post-infection birds had recovered the same liveweight for age as the uninfected birds. Similar benefits were seen with respect to feed intake, feed conversion ratio, and faecal blood scores.

How exactly can such 'micro-doses' provide such 'macro-performance' benefits? Many independent studies with these plant metabolites have reported on their antioxidant, anti-inflammatory and immune stimulating properties, and it is believed that these are the underlying modes of action for the benefits seen at the level of the gut. This was supported by a follow-up trial



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FIGURE 1 - EFFECTS ON LIVEWEIGHT AFTER FEEDING NE AT INCREASING DOSAGES TO EIMERIA ACERVULINA-INFECTED BROILERS

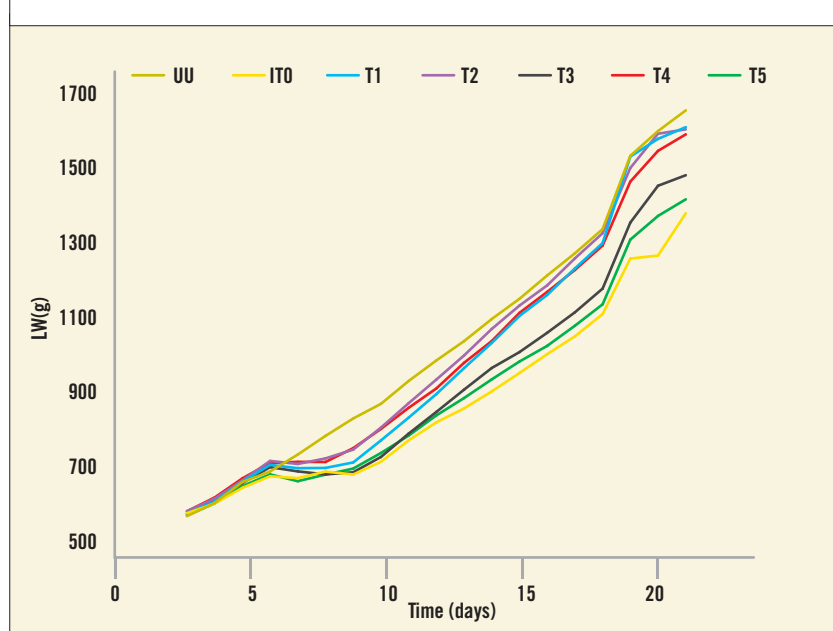


FIGURE 2 - EFFECTS ON INTESTINAL INTEGRITY FROM FEEDING NE TO BROILERS INFECTED WITH *E. ACERVULINA*

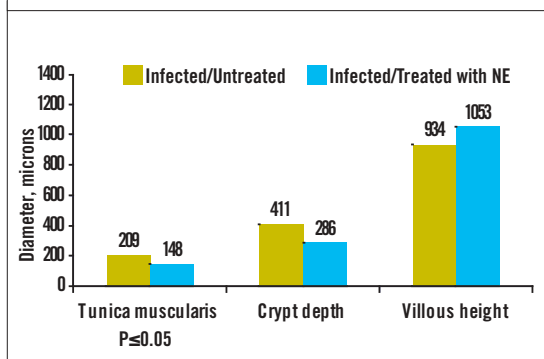
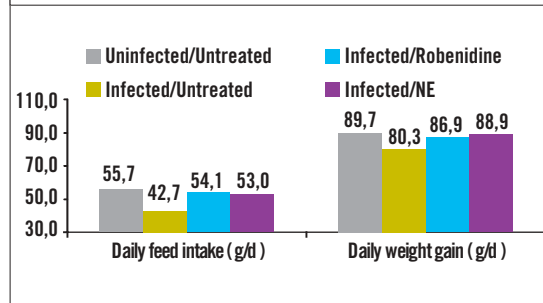


FIGURE 3 - EFFECTS ON DAILY FEED INTAKE AND DAILY WEIGHT GAIN FROM SUPPLEMENTING NE VERSUS ROBENIDINE HYDROCHLORIDE IN BROILERS INFECTED WITH *EIMERIA* OOCYSTS



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conducted at the University of Leeds with broiler chickens infected with 50,000 sporulating *E. acervulina* oocysts at 28 days of age and fed either a commercial diet with or without the supplementation with the 1:1 thymol:carvacrol combination (NE). Seven days post-infection, intestinal morphology was determined. Addition of NE resulted in improved intestinal integrity as measured by decreased tunica muscularis thickness and duodenal crypt depth (Figure 2), leading to the conclusion that this combination possibly acts by reducing the impact of coccidiosis on intestinal integrity. These underlying effects yield benefits at the performance level in broilers similar to classical coccidiostats. A 42-day farm trial was conducted in April, 2006 with the 1:1 thymol:carvacrol combination (NE) at 62.5 g/t versus robenidine hydrochloride and a negative control in broilers infected with a mixture of equal parts of *E. acervulina*, *E. maxima* and *E. tenella* applied through the drinking water at 300,000 sporulating oocysts at day 17. As shown in Figure 3, the application of the 1:1 thymol:carvacrol combination (NE) in the feed improved animal feed intake by 9%, and daily weight gain by 17% compared to the negative control group infected with the *Eimeria* inoculant and without additive. These results illustrate the benefits of NE on reducing the impact of the anorexic effects from *Eimeria* parasitism.

ENHANCEMENT WITH ENCAPSULATION

With the formulation at the core, fully exploiting the bioactive properties of plant extracts and benefits on animal performance requires serious consideration about methods of delivery. Arguably, the extent to which plants and their extracts can be exploited is dependent upon the degree to which dosage can be controlled, and this is dependent upon the stability and kinetics of the material to be delivered, and the method of delivery itself.

While the choice of individual phytomolecules is of primary interest, their administration in a sustainable, controlled fashion is also important. Even though the concepts of microencapsulation and controlled release are relatively old, attention to these concepts with respect to plant extracts has been only recent. A patent-pending process developed in Tarragona, Spain has shown the benefits for the accurate and controlled delivery of phytomolecules to the target site. This method includes a two-step encapsulation process which is highlighted by two unique physical characteristics: first, the concentration of up to 57% of active material in a single particle and second, the full encapsulation of this particle by a special layer of mono- and di-glycerides which assures that all active components are retained below the surface. Nowhere are these benefits more evident than with thymol and carvacrol. Without this protection system, studies under pelleting conditions have shown that up to 25% of thymol and carvacrol are lost either through volatilisation or oxidation. With the addition of the protective layer, these substances are retained upon pelleting. Furthermore, fluidity and dispersibility have been shown to be improved. The added value of this protection system is the assurance that a consistent, stable dose of active material is delivered to the animal, and once inside the animal is released, and not before.

NOT ONLY FOR BIRDS

The benefits of the protection system have been used to improve the delivery of thymol and carvacrol into calf feeding systems. The benefits on product stability, fluidity, and palatability, along with the potential for post-gastric delivery to the lower intestine where *Eimeria* reside, are the focus of a series of field studies being conducted in calves. The first of these results are shown in Figure 4, where the combination of thymol and

FIGURE 4 - EFFECTS ON ANIMAL LIVE WEIGHT, DAILY WEIGHT GAIN, FAECAL SCORE, AND OOCYST OUTPUT FROM FEEDING NE TO CALVES INFECTED WITH EIMERIA OOCYSTS

	Control diet with sodium monensin	Control diet with NE ¹ at 125 g/t	Control diet with NE ¹ at 250 g/t
Starting weight	71.0	73.1	74.8
Final weight	157.3	158.1	152.7
Daily weight gain (kg)	1.03 ^b	1.01 ^a	0.93 ^a
Faecal score ²	2.5 ^b	1.8 ^a	2.2 ^{ab}
Treatment score ³	1	2	1
Total oocysts / g	1258 ^a	1844 ^{ab}	988 ^b
Sporulating oocysts %	35%	47%	28%

a, ab, b: Means with different letters are significantly different ($p \leq 0,05$).

¹ NE: 1:1 equivalents of thymol and carvacrol, enhanced by patent-pending encapsulation process

² Faecal score: 1 = normal, 2 = foamy, 3 = mucousy, 4 = sticky, 5 = hard

³ Treatment score (number of animals pulled for treatment)



Photo at 40X magnification of the 1:1 combination thymol: carvacrol enhanced by a patent-pending encapsulation process (Carotenoid Technologies, Tarragona, Spain) to protect against loss of active ingredients, palatability problems and potential release to the lower gut.

carvacrol was fed in a pelleted calf starter diet (dietary energy 15.3 MJ/kg, dry matter 93%, crude protein 16%) with alfalfa hay to dairy calves at two different dosages versus a control. Not only did application of the 1:1 combination of thymol and carvacrol have benefits on daily weight gain as seen in broilers, but it also reduced oocyst output in a dose-dependent manner. At the lower dose, liveweight and daily weight gain were improved, while at the higher level, intestinal integrity as measured by faecal score and oocyst output were most significantly affected. At both the feeding levels tested, results approached the level of the positive control sodium monensin at its recommended dose.

THE NEXT STRATEGY FOR *EIMERIA*

The European in-feed antibiotic ban which came into effect earlier this year has led to the scrutiny of the short- and long-term risks of other conventional feed additives such as chemical coccidiostats. Many progressive livestock operations have discovered that putting more emphasis on intestinal health can help young animals combat enteric challenges from *Eimeria* and reduce or eliminate the need for chemical additives. Improving intestinal health can potentially be achieved without the use of chemical additives. Plant extracts, as evidenced by this investigative project at the University of Leeds, may provide alternative cost-effective solutions in the fight against intestinal *Eimeria* spp. parasitism. <-

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