

*The effect of natural alternatives for anti-microbial growth promoters in broiler diets*

L.A. den Hartog<sup>1\*</sup>, A. Gutiérrez del Álamo Oms<sup>2</sup>, J. Doorenbos<sup>3</sup>, A. Flores Miñambres<sup>2</sup>.

<sup>1</sup>Nutreco Agriculture Research & Development P.O Box 220, 5830 AE Boxmeer, The Netherlands. E-mail: [leo.den.hartog@nutreco.com](mailto:leo.den.hartog@nutreco.com). <sup>2</sup>Nutreco Poultry and Rabbit Research Centre, Ctra cm 4004, km 10.5, 45950 Casarrubios del Monte (Toledo) Spain. <sup>3</sup>HendrixUTD, P.O Box 220, 5830 AE Boxmeer, The Netherlands.

**Abstract**

From the first of January, 2006, the use of all anti-microbial growth promoters (AMGP's) within the European Union will be banned. For this reason a lot of research dedicated to testing promising natural alternatives for AMGP's has been performed.

There are several ways to test the effectiveness of alternative products, both *in vitro* and *in vivo*. Under experimental conditions it has been shown that a controlled challenge of broilers with a chyme homogenate obtained from birds suffering from malabsorption syndrome (MAS) is a reliable model to determine the effectiveness of these products.

In the last years many products have been tested in this challenge model. Based on the results of these trials it is concluded that yeast  $\beta$ -glucans are an effective alternative during the starter period for birds suffering from digestive disorders. Also a mix between medium chain fatty acids and organic acids is a good alternative for anti-microbial growth promoters.

**Introduction**

From the first of January, 2006, the use of all anti-microbial growth promoters (AMGP's) in poultry diets produced within the European Union will be banned. For this reason a lot of research dedicated to testing promising natural alternatives for AMGP's has been performed during the last years.

The aim of adding a product can be twofold, either to maintain or increase technical performance in general (without any specific action against pathogens), or to be able to control the infection pressure caused by a specific pathogen (i.e. like *Clostridium Perfringens*, *Salmonella* or *Escherichia Coli*).

**Test models for natural alternatives for AMGP's**

Normally a first screening of products is performed under well-controlled experimental conditions (either or not preceded by an *in vitro* selection to make sure that a product will be effective anyhow when activity against a certain pathogen is required). Generally, these experimental conditions are developed in such a way that performance of the bird is higher than what is seen in commercial practice, for example because of a lower number of animals per m<sup>2</sup> or m<sup>3</sup> in the house, because of a lower infection pressure or because of the higher amount of personnel taking care of the birds. This will make it more difficult to observe any effect of a tested product, or to extrapolate results from trials conducted under experimental conditions towards commercial practice. An opportunity to overcome this problem is to develop a challenge model so that the conditions and therefore the performance of the birds will resemble more practical conditions.

An example of such a model is to incorporate a highly viscous material like carboxy methyl cellulose (CMC) into the diet of the birds. It has been shown that adding CMC to the diet increases microbial activity in the small intestine of broilers (Smits, 1996). This increase is undesirable because it will lead to more competition for nutrients between the microbes and the host. Also nutrient digestibility (mainly of the fat fraction in the diet) will be impaired when microbial activity is increased. For these reasons lower animal performance (in terms of average daily gain (ADG), average daily feed intake (ADFI) or feed conversion ratio (FCR))

will be observed. The objective of a CMC model is to determine if addition of certain natural alternatives can overcome the negative effects of CMC added to broiler diets, and thereby give an indication of the effectiveness of these products against the intestinal flora.

Another model that can be used is to infect birds with a controlled dosis of a chyme homogenate obtained from birds suffering from malabsorption syndrome (MAS). The objective of such a model is not to study the MAS infection itself or to try to solve the MAS problem, but it should serve as a model for flocks with intestinal health problems. In this model the addition of a natural alternative to the diet of MAS infected birds should give a more pronounced positive effect on technical results than when given to birds kept under normal conditions.

In table 1 the difference in performance is given when comparing birds held in the CMC or the MAS model, to birds kept under normal conditions.

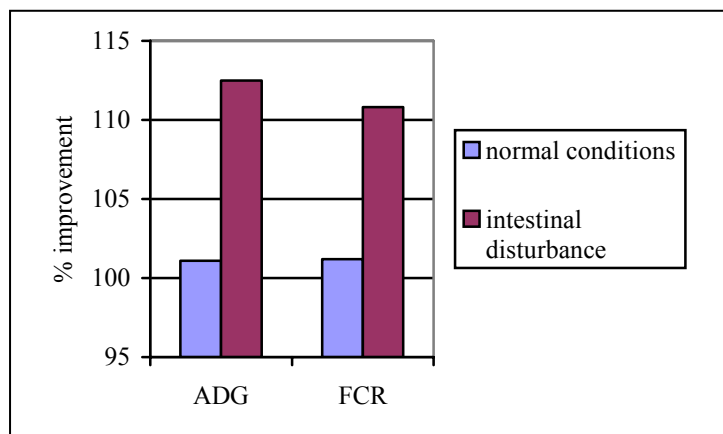
Table 1. Difference in performance between birds kept in a CMC or a MAS model, compared to birds kept under normal conditions (mean results form 3 trials (CMC model) or 30 trials (MAS model)) (trial reports Nutreco Poultry and Rabbit Research Centre 1996-2005).

	% difference with birds kept under normal conditions	
	MAS model	CMC model
Weight gain 0-21 days	10-15% lower	0-5% lower
FCR 0-21 days	3-4% higher	4-7% higher
Weight gain 0-42 days	5-8% lower	2-4% lower
FCR 0-42 days	1-2% higher	4-7% higher
Mortality	No effect	Tended to increase (not consistent between trials)

Normally intestinal infections show most of the problems in the beginning of the infection, and later on some compensation in technical performance is observed, as is seen with the MAS model. The CMC model shows a more constant effect on weight gain and feed conversion. It seems that the effect of CMC on FCR is mainly caused by physical effects of the CMC, which is confirmed by the increased feed intake that is observed in the CMC trials, indicating that the birds try to compensate for the reduced digestibility of the feed. In the case of an infection normally feed intake is not increased, as is the case in most of the MAS trials. This indicates that the worse FCR in these trials is caused by problems with intestinal infections.

The most widespread used AMGP in broiler diets is Avilamycine. In graph 1 the effect of Avilamycine in the starter diet on the technical results of broilers kept under normal conditions or challenged with MAS is given, as percentage improvement compared to a negative control.

Graph 1. Effect of Avilamycine on technical performances of broiler chickens kept under control or challenged conditions.



It can be observed that in the non-infected birds kept under normal experimental conditions Avilamycine does not lead to a consistent positive effect on technical results (average improvement of 1% with a range of 0 to 5%), while in birds suffering from intestinal disturbances there is a clear improvement.

### Materials and Methods

Most of the results of the experiments discussed in this paper are based on trials performed with birds kept both under our normal experimental conditions (control) and MAS-challenged conditions (challenged). This will give the possibility to draw conclusions about the effectiveness of a product when given to birds that are kept under good conditions and management, as well as for birds that are suffering from digestive problems.

Trials have been performed in 2 identical rooms, in which in 1 of the rooms day-old birds were orally challenged with a standard dosis of a MAS homogenate. Feed and water were provided *ad libitum*.

A negative control group (without any AMGP) was used in each trial to allow relative comparisons of products between trials. Depending on the number of products tested, there were 12 or 16 replicates per treatment. One replicate consists of a pen of 11 birds.

### Results and Discussion

There are many natural alternatives for AMGP's. The final goal of all the alternatives is a) to provide the host with more available nutrients and b) to reduce the metabolic demands for maintaining the absorptive and immunological function of the gastro-intestinal tract. However, the way each product works tends to be very different. Some are immune stimulating products (e.g.  $\beta$ -glucans), others are preventing pathogens from attaching to the intestinal lining (e.g. mannanoligosaccharides) or have an antibacterial effect (e.g. medium chain fatty acids).

During the last years many natural alternatives have been tested at the Nutreco Poultry and Rabbit Research Centre (PRRC). In this paper, trial results with some of these products are presented. These products can be grouped into different classes:

- Immune stimulating products
- Herbal extracts and etheric oils
- Short and medium chain fatty acids
- Organic acids

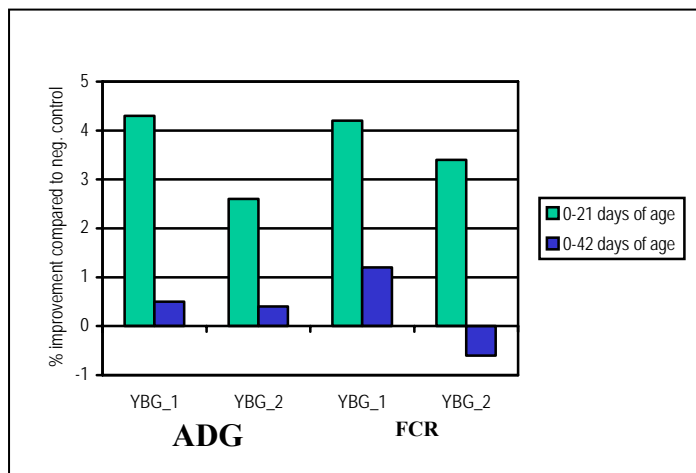
## 1. Effect of Yeast $\beta$ -D-glucans

Yeast  $\beta$ -D-glucan is a polyglucose polysaccharide derived from the cell walls of baker's yeast or *Saccharomyces cerevisiae*. Usually referred to as yeast  $\beta$ -glucan, it consists of straight-chain and branched polymers. The straight-chain structures are (1,3)- $\beta$ -D-linked glucose polymers and (1,6)- $\beta$ -D-linked glucose polymers. The branched polymers consist of a (1,3)- $\beta$ -D-linked backbone containing varying degrees of (1,6)- $\beta$  branches.

The mode of action of  $\beta$ -glucans consists of stimulating the immune system: there is a specific receptor for beta-1,3-glucan on the surface of certain cells, called macrophages that, when activated, stimulate the immune system which produces the cells to combat an infection (Williams et al., 1996).

Yeast  $\beta$ -glucans were tested at the NPRRC under control and challenged conditions. While under control conditions yeast  $\beta$ -glucans did not create differences compared to the diet without any growth promoter (data not shown), under challenged conditions yeast  $\beta$ -glucans improved ADG and FCR during the first three weeks after infection; there after, the effects were smaller (Graph 2).

Graph 2. Effect of two different yeast  $\beta$ -glucans (YBG\_1 and YBG\_2) on technical results of challenged broilers. Nutreco PRRC-2001.



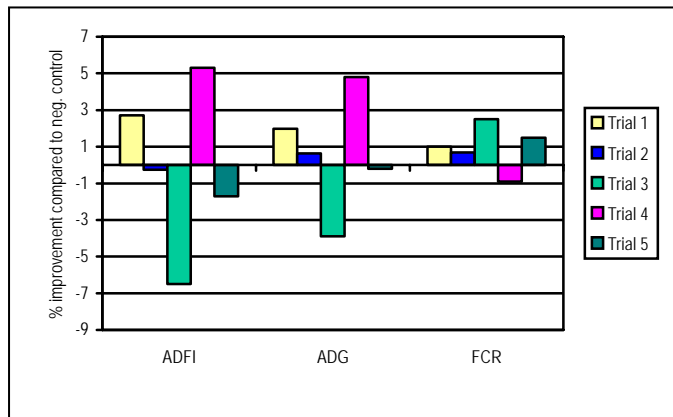
Based on the trials performed it can be concluded that yeast  $\beta$ -glucans are effective during the starter period (first three weeks) and when animals are suffering from digestive disorders.

## 2. Herbal extracts and etheric oils

Herbs and plant extracts are natural alternatives and possess *in vitro* antibacterial and antifungal activity (Hammer et al., 1999). Different publications provide information about whether or not a plant extract possesses activity against Gram-positive and Gram-negative bacteria and fungi. However, there is not a lot of information available about the extent or spectrum of this activity.

Inside the NPRRC, plant extracts and etheric oils were widely studied in at least 15 trials. Results obtained were not consistent. Products were tested in several trials through the whole year and different and not consistent responses were observed. An example of the results of a herbal extract tested in 5 different trials is presented in graph 3.

Graph 3. Effect of one herbal extract fed during the starter period, on technical results of challenged broilers (results from 5 trials). Nutreco PRRC-1998, 2000 and 2002.



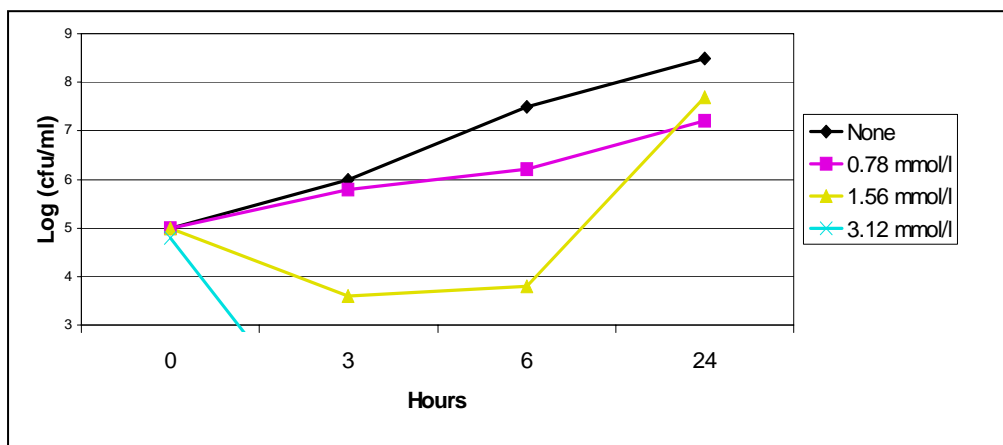
The application of herbal extracts and etheric oils in broiler diets does not seem to have a consistent positive effect, especially when animals are suffering from digestive problems.

### 3. Short and medium chain fatty acids

The possible mode of action of short and medium chain fatty acids in a situation where digestibility is compromised can be twofold. In the first place, from Nutreco *in vitro* research it has been found that medium chain fatty acids (caproic, caprylic and capric acid) can inhibit certain pathogenic bacteria like *E. coli* and *Cl. perfringens*. They could therefore have a positive effect on the microflora. A second mode of action extensively described in literature are the good digestibility properties of MCFA. Because of the short chain length, they are more easily digested and are therefore used in human clinical nutrition. Also in a situation of digestive disorders they might have a positive effect, because fat digestion is much lower.

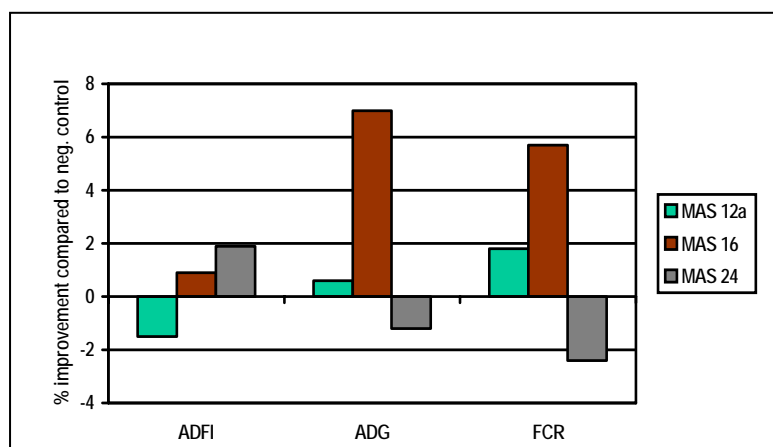
An *in vitro* test measuring *E. coli* growth at 3, 6 and 24 hours was performed at ID-Lelystad (Graph 4). In total 4 different concentrations of MCFA were tested (0, 0.78, 1.56 and 3.12 mmol/l). A reduction in the pathogenic *E. coli* strain was observed when MCFA were added to the growth media with the best results observed with 3.12 mmol/l of MCFA added.

Graph 4. Inhibition of a pathogenic *E. coli* strain using different concentrations of medium chain fatty acids. *In vitro* test. ID-Lelystad 2001.



The promising results observed in the *in vitro* research were evaluated in several *in vivo* trials (Graph 5). Different doses of MCFA were tested (0.05%, 0.1% and 0.15% in trials MAS-24, MAS-12a and MAS 16 respectively). No positive effects of adding MCFA at a low level (0.05%) were observed. When doses of 0.1% or 0.15% were used, positive effects on daily gain and feed conversion rate were observed. The 0.15% inclusion level seemed the optimal dose to be added (Graph 5).

Graph 5. Effect of Medium Chain Fatty Acids on technical results of challenged broilers (MAS-12a, MAS-16 and MAS-24). Nutreco PRRC-2002



Based on the trials presented in this article it can be concluded that MCFA could be considered as an alternative for AMGP's especially when animals are suffering from intestinal disturbances.

#### 4. Organic acids

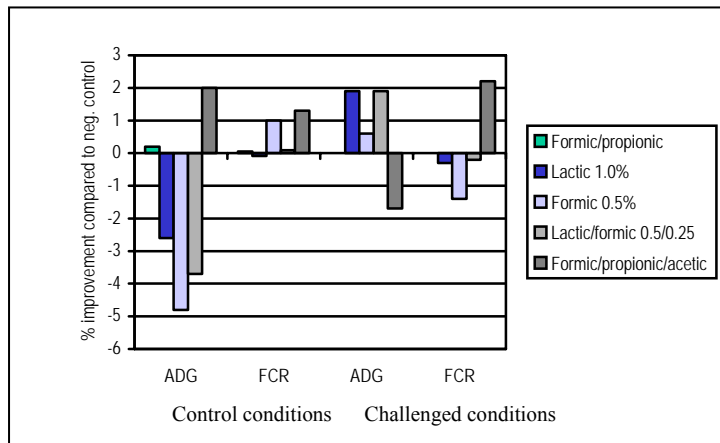
Organic acids are widely distributed in nature as normal constituents of plants or animal tissues. They are also formed through microbial fermentation of carbohydrates predominantly in the large intestine of poultry.

Rather than dietary acidifiers, organic acids are better known as effective preservatives. Their primary antimicrobial action (selective growth inhibition or delay) is through pH depression of the diet and the drinking water (as a rather *in vitro* activity).

*In vivo* action is mainly based on the same mechanism: acidification of the stomach with favourable effects on pepsinogen- pepsin conversion. However, more important is the ability of organic acids to change from an undissociated to a dissociated form, depending on the environmental pH, making them effective antimicrobial agents. When an acid is in its undissociated form it can freely diffuse through the semipermeable membrane of the microorganisms into their cell cytoplasm. Once in the cell, where the pH is maintained near 7, the acid will dissociate and suppress cell enzymes and nutrient transport systems. The efficacy of an acid in inhibiting microbes is dependent on its pKa value, which is the pH at which 50% of the acids are dissociated. Organic acids with higher pKa values are more effective preservatives and their antimicrobial efficacy is generally improved with increasing chain length and degree of insaturation.

Trials done at Nutreco PRRC showed different results depending on the conditions in which animals were grown (Graph 6). Animals grown under control conditions and fed with organic acids improved their feed conversion rate while challenged birds tend to increase their daily gain and to have a higher feed conversion rate.

Graph 6. Effect of organic acids on technical performances of broilers kept under control or challenged conditions, from 0 to 21 days of age. Nutreco PRRC-2000.

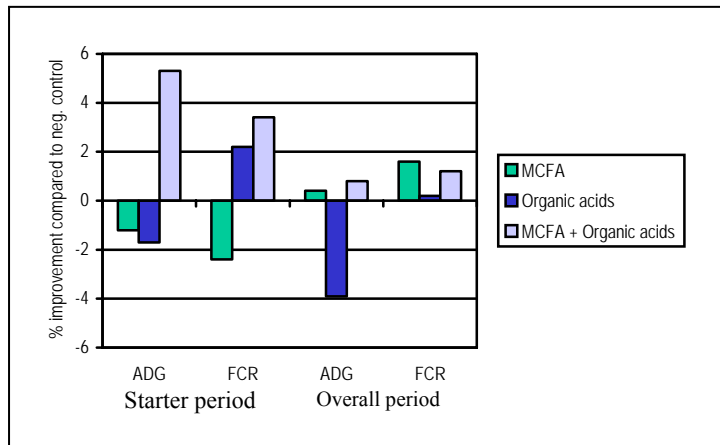


### 5. Synergetic effects of MCFA and organic acids

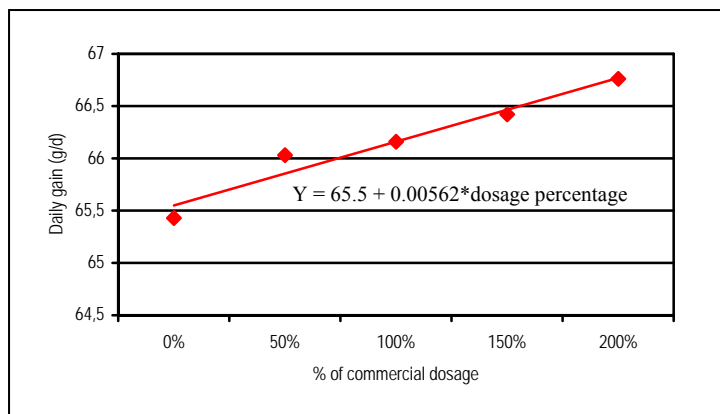
Because of the mode of action of organic acids and medium-chain fatty acids it is believed that the combination of these products can have synergetic effects in the gastro-intestinal tract of broilers. It is expected that the MCFA's will have an effect on the integrity of the bacterial cellwall, thereby facilitating the penetration of the organic acid into the bacteria itself where it can develop its anti-microbial activity.

Two trials to test the synergetic effect of MCFA and organic acids were performed at the Nutreco PRRC. In trial one the effect of the addition of organic acids (0.325%) either or not in combination with MCFA (0.05%) was tested under challenged conditions (Graph 7). The combination of medium chain fatty acids and organic acids resulted in higher daily gain and better feed conversion rate when animal were challenged. This effect was more pronounced during the starter period and less for the overall period. In the second trial the effect of increasing the recommended dosage of a commercial product based on organic acids and MCFA was tested in broilers kept under control conditions (Graph 8). A linear response was observed for daily gain when the inclusion level of the product was increased from 50% to 200% of the recommended dosage.

Graph 7. Effect of organic acids, either or not in combination with MCFA, on technical performances of challenged broilers from 0 to 20 (starter period) and 0 to 40 (overall period) days of age. Nutreco PRRC-2003



Graph 8. Effect of increasing the dosage of a commercial product based on organic acids and MCFA on technical performances of male broilers. Animals grown under control conditions. Nutreco PRRC-2004.



Based on the trials performed at Nutreco PRRC, the combination of organic acids and MCFA can be a good alternative for anti-microbial growth promoters.

## Conclusions

Trials presented in this paper are performed with both, non-challenged and challenged animals.

From all the products and based on these trials it can be concluded that the mix between medium chain fatty acids and organic acids is a good alternative for anti-microbial growth promoters.

## References

- CUMMINGS, J.H. and MACFARLANE, G.T.** (2002). Gastrointestinal effects of Prebiotics. *British Journal of Nutrition* 87 Suppl 2:S145-S151.
- GHADBAN, G.S.** (2002). Probiotics in broiler production – a review. *Archiv. für Geflügelkunde* 66: 49-58.

**HAMMER, K.A., CARSON, C.F. and RILEY T.V.** (1999). Antimicrobial effect of essential oils and other plant extracts. *J. Appl. Microbiol.* 86: 985-990.

**ID-LELYSTAD** (2001). Inhibition of pathogenic E. coli strain. Personal communication.

**NUTRECO PRRC** (1998). Alternative products for feed savers in broiler chicken diets. Internal report PB-94b.

**NUTRECO PRRC** (2000). The effect of supplemental medium chain fatty acids on zootechnical performance of broilers either or not infected with malabsorption syndrome. Internal report MAS-12a.

**NUTRECO PRRC** (2000). Effect of etheric oils products on technical performance of broilers. Internal report PB-104.

**NUTRECO PRRC** (2000). The effect of lactic and/or formic acid on zootechnical performance of broilers either or not infected with malabsorption syndrome. Internal report MAS-12b.

**NUTRECO PRRC** (2001). The effect of potential immune stimulating additives on technical performance in MAS infected broilers. Internal report MAS-13.

**NUTRECO PRRC** (2001). The effect of Medium chain fatty acids on performance and microflora parameters of broilers, either or not infected with Malabsorption syndrome (MAS). Internal report MAS-16.

**NUTRECO PRRC** (2002). The effect of different etheric oils on performance of broiler chickens. Internal report NPB-17.

**NUTRECO PRRC** (2002). The effect of an experimental product with Greenline components on performance of broilers, either or not infected with MAS – second trial. Internal report MAS-20.

**NUTRECO PRRC** (2003). The effect of different yeast cell wall products as possible Greenline components on technical performance of normal or MAS-infected broilers. Internal report MAS-23.

**NUTRECO PRRC** (2003). The effect of organic acids, either or not in combination with MCFA on technical performance of normal or MAS-infected broilers. Internal report MAS-24.

**NUTRECO PRRC** (2004). The effect of herbal extracts on technical performance of normal or MAS-infected broilers. Internal report MAS-26.

**NUTRECO PRRC** (2004). The effect of different dosages of a combination of organic acids and MCFA on technical performances of male broilers. Internal report NPB-28.

**SMITS, C.H.M.** (1996). Viscosity of dietary fibre in relation to lipid digestibility in broiler chickens. *PhD Thesis* Wageningen University, the Netherlands, 140 pp.

**SPRING, P., WENK, C., DAWSON, K.A. and NEWMAN, K.E.** (2000). The effects of dietary mannanoligosaccharides on cecal parameters and the concentrations of enteric bacteria in the ceca of Salmonella-challenged broiler chicks. *Poultry Science* 79: 205-211.

**WILLIAMS D.L., MUELLER A., BROWDER W.** (1996). Glucan-based macrophage stimulators: a review of their anti-infective potential. *Clin Immunother.* 5:392-399.