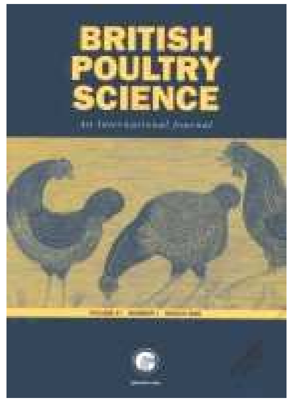
# Influence of whole wheat inclusion and a blend of essential oils on the performance, nutrient utilisation, digestive tract development and ileal microbiota profile of broiler chickens

Ahmed M Amerah, Alexandre Péron, Fifi Zaefarian, Velmurugu Ravindran






# Influence of whole wheat inclusion and a blend of essential oils on the performance, nutrient utilisation, digestive tract development and ileal microbiota profile of broiler chickens

| | |
|---|---|
| Journal: | *British Poultry Science* |
| Manuscript ID: | CBPS-2010-077.R1 |
| Manuscript Type: | Original Manuscript |
| Date Submitted by the Author: | 05-Aug-2010 |
| Complete List of Authors: | Amerah, Ahmed; Danisco Animal Nutrition<br>Péron, Alexandre; Danisco Animal Nutrition<br>Zaefarian, Fifi; Massey University, Institute of Food, Nutrition and Human Health<br>Ravindran, Velmurugu; Massey University, Institute of Food, Nutrition and Human Health |
| Keywords: | Broilers, Nutrition, Plants |
| | |

CBPS-2010-077

Edited Lewis September 2010, MacLeod December 2010

# Influence of whole wheat inclusion and a blend of essential oils on the performance, nutrient utilisation, digestive tract development and ileal microbiota profile of broiler chickens

A.M. AMERAH, A. PÉRON, F. ZAEFARIAN[1] AND V. RAVINDRAN[1]

*Danisco Animal Nutrition, Marlborough, SN8 1XN, UK and [1]Institute of Food, Nutrition and Human Health, Massey University, New Zealand.*



Running title: Whole wheat and essential oils

**Correspondence to:** Ahmed Amerah, Danisco Animal Nutrition, Marlborough, SN8 1XN, UK

E-mail: Ahmed.amerah@danisco.com

Accepted for publication 12th August 2010

**Abstract** 1. The aim of the present experiment was to examine the influence of whole wheat inclusion and a blend of essential oils (EO; cinnamaldehyde and thymol) supplementation on the performance, nutrient utilisation, digestive tract development and ileal microbiota profile of broiler chickens.

2. The experimental design was a 2 x 2 factorial arrangement of treatments evaluating two wheat forms (ground wheat [GW] and whole wheat [WW]; 100 and 200 g/kg WW replacing GW during starter [1 to 21 d] and finisher [22 to 35 d] diets respectively) and two levels of EO inclusion (0 or 100 g/tonne diet). All dietary treatments were supplemented with 2000 xylanase units/kg feed. Broiler starter and finisher diets based on wheat and soybean meal were formulated and each diet fed *ad libitum* to 6 pens of 8 male broilers.

3. During the trial period (1-35 d), wheat form had no significant effect on weight gain or feed intake. However, WW inclusion tended to improve feed per gain. Essential oil supplementation significant improved weight gain in both diets, but the improvements were greater in the GW diet as indicated by a significant wheat form x EO interaction.

4. Main effects of wheat form and EO on the relative weight, length and digesta content of various segments of the digestive tract were not significant. Significant interactions, however, were found for relative gizzard and caecal weights. Essential oil supplementation significantly increased the relative gizzard weight and lowered relative caecal weight in birds fed on the GW based diet, but had no effect in those fed on the WW based diet.

5. Whole wheat inclusion and EO supplementation significantly improved apparent ileal nitrogen digestibility. Apparent ileal digestible energy was not significantly influenced by the dietary treatments.

6. Ileal microbiota profiling, using denaturing gradient gel electrophoresis, showed that the ileal microbiota composition was influenced by feed form. The mean numbers of bacterial

species in the ileal contents of birds fed on the GW diet supplemented with EO tended to be higher than those of the ileal contents of birds fed on unsupplemented GW based diet.

7. The present data suggested that dietary addition of EO improves broiler weight gain and ileal nitrogen digestibility both in GW and WW based diets, but that the magnitude of the response to EO for weight gain was greater in GW based diet. Whole wheat feeding was found to be beneficial in terms of feed efficiency.

## INTRODUCTION

The need to reduce feed costs has renewed the interest in the poultry industry in whole grain feeding, a common practice in the early days of commercial poultry production. There appears to be a general consensus that the inclusion of whole grain in broiler diets, using mixed feeding technique, will have no adverse effect on broiler performance (Preston *et al.,* 2000; Plavnik *et al.*, 2002; Amerah and Ravindran, 2008). It has been suggested that the beneficial effects of whole grain feeding on nutrient utilisation may be related to the positive effect of whole grain on gizzard development (Ferket, 2000; Svihus and Hetland, 2001). A more developed gizzard is associated with increased grinding activity, resulting in increased gut motility and a greater digestion of nutrients (Amerah *et al.*, 2007*a*). Higher gizzard functionality may also play a positive role in the control of bacterial populations in the gut and on gut health (Bjerrum *et al.*, 2005).

Essential oils (EO) are steam-volatile or organic-solvent extracts of plants, usually used for the pleasant odour of their essence, their flavour, their antiseptic or preservative properties, as well as their antimicrobial effect (Burt, 2004; Lee *et al.*, 2004; Wallace, 2004). The antimicrobial properties of EO have encouraged their use as a natural replacement for antibiotic growth promoters in animal feeds. In addition to the positive effects of EO against the colonisation and proliferation of pathogenic bacteria (Lee *et al.*, 2004), EO have been shown to improve nutrient digestibility and broiler performance (Hernandez *et al*., 2004; Lee *et al*., 2004; Cao *et al*., 2009).

The ban on the use of antibiotic growth promoters in feeds has encouraged nutritionists to explore alternative feed management strategies to improve the intestinal health and digestive efficiency of broiler chickens (Amerah *et al*., 2009*a*). Promotion of gizzard development by whole wheat (WW) feeding is one of the strategies that have been found to prevent potentially pathogenic bacteria from entering the intestinal tract (Engberg *et al*., 2004). Recent studies have highlighted the potential benefit of combining EO and carbohydrase enzymes (Cao *et al*., 2009; Péron *et al*., 2009). However, combining the effects of whole wheat inclusion on gut health and the anti-microbial properties of EO, with possible complementary improvement in broiler performance, has not yet been investigated. Therefore, the present study investigated the effects of WW inclusion and a specific blend of EO supplementation on the performance, nutrient utilisation, digestive tract parameters and ileal microbiota profile of broilers.

## MATERIALS AND METHODS

**Birds and housing**

Experimental procedures were conducted in accordance with the Massey University Animal Ethics Committee guidelines. Day-old male broilers (Ross 308) were obtained from a commercial hatchery, individually weighed and assigned to 24 cages (8 birds per cage) in electrically heated battery brooders so that the average bird weight was similar for each cage. The birds were transferred to grower cages on d 12. The battery brooders and grower cages were housed in an environmentally controlled room with 20 h of fluorescent illumination daily. The temperature was maintained at 31°C on d 1 and gradually reduced to 22°C by 21 d of age.

**Diets and conduct of the trial**

The basal diets were based on wheat and soybean meal (Table 1). Two sets of basal diets were used: one for broiler starters (1–21 d) and the other for broiler finishers (22–35 d). The basal diets based either on ground wheat (GW) or WW (100 and 200 g/kg WW replacing GW during starter (1 to 21 d) and finisher (22 to 35 d) diets respectively). Diets were formulated to meet or exceed the Ross 308 strain recommendations for major nutrients for broilers. The basal diets were supplemented with a commercial xylanase (Danisco Xylanase, Danisco Animal Nutrition, Marlborough, UK), which provided 2000 xylanase units/kg feed. The enzyme preparation is based on endo 1,4 beta-xylanase because its main activity is produced by *Trichoderma reesei*. WW was ground in a hammer mill (Bisley's Farm Machinery, Auckland, New Zealand) to pass through a screen size of 3 mm. Half of each formulated diet was supplemented with an encapsulated blend of EO (100 g/tonne; Enviva™ EO 101 G, Danisco Animal Nutrition, Marlborough, UK) and the other half was not supplemented with EO. The two active ingredients in the EO product were Cinnamaldehyde and Thymol. Titanium oxide (0.3%) was included in all diets as a dietary marker.

Table 1 near here

Each of the 4 dietary treatments was fed *ad libitum* to 6 replicate cages. Water was freely available throughout the trial. The diets were cold-pelleted (65–70°C). Body weight and feed intake were recorded at weekly intervals, and mortality was recorded daily, for each cage. Any bird that died was weighed and feed per gain values were calculated by dividing total feed intake by weight gain of live plus dead birds.

**Digestive tract measurements**

On d 35, two birds from each replicate cage were randomly selected, weighed and killed by cervical dislocation. The body cavity was immediately opened and the full and empty weights of each digestive tract segment from proventriculus to caeca were recorded. The length of each intestinal segment was determined with a flexible tape on a glass surface to prevent inadvertent stretching. The length (± 0.1 mm) of the duodenum (from the pyloric junction to the distal most point of insertion of the duodenal mesentery), the length of the jejunum (from the distal most point of insertion of the duodenal mesentery to the junction with Meckel's diverticulum), the length of the ileum (from the junction with Meckel's diverticulum to ileo-caecal junction) and caeca (the sum of the lengths from ostium to tip of each caeca) were also determined. Following division and freeing of each of these components from any adherent mesentery, their full and empty weights (± 0.1 g) were determined along with those of the proventriculus and gizzard. Digesta were removed from the gut component by gentle squeezing for empty weight determination.

**Ileal digesta collection**

On d 35, 4 more birds per replicate were killed by intravenous injection with sodium pentobarbitone. Immediately after euthanasia, the ileum was excised and divided into two parts, the anterior and posterior ileum. The ileum was defined as the portion of the small intestine extending from Meckel's diverticulum to a point 40 mm proximal to the ileo-caecal junction. Contents of the posterior ileum were collected by gently flushing with distilled water into plastic containers. Digesta were pooled within a cage, freeze-dried, ground to pass through a 0.5 mm screen size and stored at -4°C in airtight containers until laboratory analysis.

**Isolation of DNA and PCR-denaturing gradient gel electrophoresis (DGGE) analysis**

On d 35, two birds from each replicate cage were killed by intravenous injection of sodium pentobarbitone. Approximately 1ml digesta was obtained from the middle part of the ileum and stored at -80ºC until DNA extraction. Isolation of DNA and PCR-DGGE analysis were performed as described by Amerah *et al*. (2009*a*).

Diversity computed as the Simpsons diversity index, using the area under the densiometric curve of each band (projected on a Gaussian basis), represened population density of a particular species (band). Comparison of samples was based on the Dice similarity coefficient and the un-weighted pair group method using arithmetic means for clustering (Hume *et al*., 2003). The relative similarities of the groups were determined by comparing the patterns of a relatedness tree (dendogram). The degree of similarity was indicated by the percentage coefficient bar located below each dendogram. Values between 0 and 1 represented an arithmetic determination of the degree to which banding patterns were similar.

**Chemical analysis**

The dry matter (DM), nitrogen and gross energy (GE) were determined in samples of diets and excreta. Dry matter content was determined using standard procedures (AOAC, 2005, method 930.15). Nitrogen was determined by combustion (method 968.06; AOAC, 2005) using a CNS-200 carbon, nitrogen, and sulphur auto-analyser (LECO Corporation, St. Joseph, MI). Gross energy was determined using an adiabatic bomb calorimetry (Gallenkamp Autobomb, London, UK) standardised with benzoic acid. Diets and ileal digesta samples were assayed for titanium on a UV spectrophotometer following the method described by Short *et al*. (1996).

**Calculations**

Apparent ileal digestibility coefficients of nitrogen and energy were calculated using the following formula:

$$\text{Apparent ileal digestibility} = \frac{(\text{nutrient/Ti})_d - (\text{nutrient/Ti})_i}{(\text{nutrient/Ti})_d}$$

where $(\text{nutrient/Ti})_d$ = ratio of nutrient to titanium in the diet, and

$(\text{nutrient/Ti})_i$ = ratio of nutrient to titanium in the ileal digesta.

**Statistical analysis**

For performance parameters, cage means served as the experimental unit for statistical analysis. For digestive tract measurements, individual birds were considered as the experimental unit. All data were subjected to two-way ANOVA using the general linear models procedure of SAS (SAS, 1997). For the microbial profile analysis, both band number and Shannon Weiner index of diversity were normally distributed; thus the data were analysed by three-way ANOVA for the effects of wheat form, EO inclusion and gel. The latter term was introduced as, in DGGE, results vary somewhat between gels due to slight variation in the denaturing gradient from gel to gel. The 12 samples from each dietary treatment were divided randomly in a balanced manner among the three gels, having 4 random samples from each dietary treatment in a gel. The differences were considered significant at $P$ <0.05 and significant differences between means were separated by the least significant difference test.

## RESULTS

**Bird performance**

The influence of treatments on the performance of broilers is summarised in Table 2. During the starter phase (1-21 d), a significant interaction between wheat form and EO supplementation was observed for weight gain. Essential oils improved the weight gain in birds fed on GW based diet but had no effect in birds fed on the WW based diet. WW feeding had no significant effect on feed intake, but significantly improved feed per gain. Essential oil supplementation significantly increased feed intake, but had no significant effect on feed per gain. There was no significant interaction between wheat form and EO supplementation for feed intake and feed per gain.

During the finisher period (22-35 d), wheat form had significant no effect on weight gain, feed intake or feed per gain. Essential oil supplementation significantly improved weight gain both in GW and WW diets. No significant wheat form x EO interactions were observed for any of the performance parameters measured.

Over the whole trial period (1-35 d), EO supplementation significantly improved weight gain in both diets, but the magnitude of improvement was greater in birds fed on the GW diet, resulting in a significant wheat form x EO interaction. Whole wheat inclusion tended ($P$ = 0.06) to improve the feed per gain.

**Nutrient utilisation**

The influence of treatments on ileal nitrogen and energy digestibility is summarised in Table 3. Whole wheat inclusion and EO supplementation significantly improved ileal nitrogen digestibility. A tendency ($P$ = 0.06) for wheat form x EO interaction was observed for ileal energy digestibility coefficient and apparent ileal digestible energy. Essential oils supplementation tended to improve ileal energy digestibility coefficient and IDE in birds fed on the GW diet, but had no effect in those fed on the WW diet.

Tables 2 and 3 near here

**Digestive tract morphology**

The effects of wheat form and EO supplementation on the relative weight, length and digesta content of various digestive compartments are shown in Table 4. Treatments had no effect on most of the parameters measured. However, a significant interaction between wheat form and EO supplementation was found for relative gizzard and caecal weights. Essential oil supplementation increased the relative gizzard weight and reduced the caecal weight in birds fed on the GW diet, but had no effect in birds fed on the WW diet.

**Number of bands and Simpsons diversity index of diversity**

The effects of wheat form and EO supplementation on the number of bands and Simpsons diversity index of diversity are shown in Table 5. There were no significant differences in the numbers of bacterial species from the ileal microflora of birds fed on the GW diet compared with those fed on the WW diet. However, the mean numbers of bacterial species in the ileal contents of birds fed on the GW diet supplemented with EO diet tended ($P$ = 0.07) to be higher than those fed on the unsupplemented GW diet.

Tables 4 and 5 near here

**Dendographic analysis**

Dendographic analysis showed a tendency for similarity of microbial communities on a basis of feed form but not on a basis of EO supplementation. In all gels (Figures 1 to 3), the results from birds fed on ground and whole wheat diets tended to be separated, and to cluster on opposite sides of the dendogram.

Figures 1, 2 and 3 near here

DISCUSSION

In the present study, over the whole 35-d trial period, WW inclusion tended to improve feed per gain, but had no effect on the weight gain or feed intake. Published data on the effects of WW inclusion in broiler diets have been contradictory. Several studies have reported improvements in feed per gain when WW has replaced part of the ground grain (Plavnik *et al*., 2002; Wu and Ravindran, 2004; Wu *et al*., 2004; Ravindran *et al*., 2006), while others found no improvement in feed efficiency (Preston *et al*., 2000; Banfield and Forbes, 2001; Svihus *et al*., 2004; Amerah and Ravindran, 2008). In one study, feed efficiency was found to be poorer when WW substituted part of the ground wheat (Bennett *et al*., 2002). Several factors may be contributing to these inconsistent results, including differences in methodology and experimental protocols (Wu and Ravindran, 2004) or in the dietary nutrient density in the basal diets (Plavnik *et al*., 2002). Differences in wheat types/cultivars may provide a third explanation (Amerah *et al*., 2009*b*), as the wide variability in the nutrient composition (Inglett, 1974), metabolisable energy (Hughes and Choct, 1999) and nutrient digestibility (Ravindran *et al*., 2005; Carré *et al*., 2007) of wheat is well known.

In the present study, the blend of cinnamaldehyde and thymol supplementation improved weight gain both in GW and WW diets, with a greater response observed in birds fed on the ground wheat diet. Several studies have reported beneficial effects of EO on weight gain (Bento *et al*., 2008; Péron *et al*., 2009; Yang *et al*., 2009), while others reported no effect (Botsoglou *et al*., 2002; Zhang *et al*., 2005; Jang *et al*., 2007). These differences have been attributed to the type of EO used and inclusion level (Cross *et al*., 2007). Cross *et al*. (2007), comparing the effect of inclusion of 10 or 100 g/tonne of different herbs (thyme, oregano, marjoram, rosemary or yarrow), reported that dietary thyme oil or yarrow herb inclusion had the most positive effects on chick performance, while oregano herb and yarrow oil were the poorest. Hernandez *et al*. (2004) reported that an EO blend, containing oregano, cinnamon, and pepper and Labiatae extract from sage, thyme, and rosemary extracts, fed to broilers gave performance levels similar to those of the antibiotic growth promoter, Avilamycin. Improved weight gain in birds given EO in the current study may be related to the improvements in the ileal N digestibility observed with EO supplementation. The positive effect of EO on protein digestibility as well as DM digestibility has been observed in previous studies (Hernandez *et al*., 2004; Cao *et al*., 2009). The higher response of EO supplementation in the GW diet may be due to the observed tendency towards lower energy digestibility in these birds. These positive effects of EO on nutrient digestibility may be associated, in part, with their digestion-stimulating properties and antimicrobial effects (Kamel, 2001).

It was expected that WW inclusion would modulate the development of the digestive tract as this has been observed in most previous studies (Preston *et al.* 2000; Plavnik *et al.* 2002; Ravindran *et al*., 2006; Amerah and Ravindran, 2008). Surprisingly, wheat form had no effect on any of the measured parameters of the digestive tract. Most studies reported increased gizzard size with WW inclusion, however, no effect of WW inclusion on relative gizzard weight or its relative digesta content was observed in the current study. The reason for this finding is not clear, but thinner gizzard muscles were observed in birds fed on the whole wheat. Thus, WW inclusion may increase gizzard volume but cause thinner gizzard muscles. Amerah *et al*. (2007*b*), comparing mash vs. pelleted diets in broilers, reported that the gizzards of birds fed on mash or pelleted diets increased uniformly in size, there being no significant change either in the ratio of content to empty mass or in the ratio of gizzard length to breadth with diet. Another possible explanation is that the wheat used in this study was soft cultivar and did not stimulate the growth of the gizzard. Interestingly, EO supplementation increased gizzard weight in birds fed on the GW diet, but had no effect in birds fed on the WW diet. However, the interaction effect of EO and wheat form on caecal weight was less clear. When included in ground wheat based diet EO resulted a reduction in caecal weight compared with those fed on supplemented diet in whole wheat based diet. The reasons for these findings are unclear. In contrast, Hernandez *et al*. (2004) reported no effect of EO inclusion on digestive tract development.

One of the objectives of the present experiment was to investigate whether the inclusion of WW and EO supplementation influences the ileal microbial composition of broilers. The DGGE band patterns (Figures 1, 2 and 3) showed that ileal microbiota composition was influenced by feed form. Similarly, Amerah *et al*. (2009*a*) reported that ileal microbiota profiling using DGGE showed separation between birds receiving the GW-based diet and birds fed on the WW-based diet. Previous studies, using media culture techniques, have shown that WW feeding increases the intestinal counts of some *Lactobacillus* species (Engberg *et al*., 2004) and reduces the number of *Clostridium perfringens* in the intestinal tract (Engberg *et al*., 2004; Bjerrum *et al*., 2005) compared with those fed on the GW-based diets.

In this study, the mean numbers of bacterial species in ileal contents of birds fed on the GW diet supplemented with EO tended to be higher than the ileal contents of birds fed on the unsupplemented GW diet. Hence, the addition of EO to GW diet appears to have induced the growth of additional species but with no accompanying overall changes in diversity. A previous study, using the same blend of EO, reported that the EO supplementation tended to lower the proportion of *E. coli* and significantly increase the proportion of bifidobacteria in the caecal digesta (Tiihonen *et al.*, 2010). On the other hand, in the present study, EO supplementation of the WW diet showed no change in the mean numbers of bacterial species or DGGE band patterns, suggesting that the ileal microbiota composition was not influenced by EO supplementation. Whole wheat inclusion induced changes in DGGE band patterns, which may have masked the effect of EO on the microbial profile in these birds. Further studies with greater numbers of replicates are required to confirm this finding.

In conclusion, the findings of the present study suggest that this blend of cinnamaldehyde and thymol improves weight gain of broilers regardless of the wheat form, but the response may be higher in the ground wheat based diet. WW inclusion showed performance benefits during the starter phase, but did not result in a significant improvement for the overall period. Essential oil supplementation and WW feeding had a positive effect of on ileal nitrogen digestibility and an hypothesis that a combination of EO and WW will have additive effects on broiler gut microflora was also tested. Essential oil supplementation in birds fed on the WW diet showed no change in DGGE band patterns, suggesting that ileal microbiota composition was not influenced by EO supplementation in these birds.

## REFERENCES

AMERAH, A.M. & RAVINDRAN, V. (2008) Influence of method of whole wheat feeding on the performance, digestive tract development and carcass traits of broiler chickens. *Animal Feed Science and Technology,* 147: 326-339.

AMERAH, A.M., RAVINDRAN, V., LENTLE, R.G. & THOMAS, D.G. (2007*a*) Feed particle size: Implications on the digestion and performance in poultry. *World's Poultry Science Journal,* 63: 439-451.

AMERAH, A.M., RAVINDRAN, V., LENTLE, R.G. & THOMAS, D.G. (2007*b*) Influence of feed particle size and feed form on the performance, energy utilisation, digestive tract development and digesta parameters of broiler starters. *Poultry Science,* 86**:** 2615-2623.

AMERAH, A.M., RAVINDRAN, V. & LENTLE, R.G. (2009*a*) Influence of insoluble fibre and whole wheat inclusion on the performance, digestive tract development and ileal microbiota profile of broiler chickens. *British Poultry Science*, 50: 366-375.

AMERAH, A.M., RAVINDRAN, V. & LENTLE, R.G. (2009*b*) Influence of wheat hardness and xylanase supplementation on the performance, energy utilisation, digestive tract development and digesta parameters of broiler starters. *Animal Production Science*, 49: 71-78.

AOAC. (2005) Official Methods of Analysis. 18th edition. Association of Official Analytical Chemists, Washington DC, USA.

BANFIELD, M.J. & FORBES, J.M. (2001) Effects of whole wheat dilution *v.* substitution on coccidiosis in broiler chickens. *British Journal of Nutrition*, 86: 89-95.

BENNETT, C.D., CLASSEN, H.L. & RIDDELL, C. (2002) Feeding broiler chickens wheat and barley diets containing whole, ground and pelleted grain. *Poultry Science*, 81: 995-1003.

BENTO, M.H.L., VAN DER KLIS, J.D. & SCHULZE, H. (2008) Efficacy of essential oils on broiler growth performance in a semi-commercial scale facility. EAAP, 59$^{th}$ annual meeting, Vilnius, pp144.

BJERRUM, L., PEDERSEN, K. &. ENGBERG, R.M. (2005) The influence of whole wheat feeding on salmonella infection and gut flora composition in broilers. *Avian Disease,* 49: 9-15.

BOTSOGLOU, N.A., FLOROU-PANER, P., CHRISTAKI, E., FLETOURIS, D.J. & SPAIS, A.B. (2002) Effect of dietary oregano essential oil on performance of chickens and on iron-induced lipid oxidation of breast, thigh and abdominal fat tissues. *British Poultry Science,* 43: 223-230.

BURT, S. (2004) Essential oils: their antibacterial properties and potential applications in foods - a review. *International Journal of Food Microbiology,* 94: 223-253.

CAO, P.H., LI, F.D., LI, Y.F., RU, Y.J., PÉRON, A. & SCHULZE, H. (2009) Essential oils and feed enzymes improve nutrient utilization in broiler chickens fed a corn/soy-based diet reduced in calcium and phosphorus. ASAS-CAAV Pacific Rim Conference, Beijing, China, pp.14.

CARRÉ B., MIGNON-GRASTEAU S., PÉRON A., JUIN H. & BASTIANELLI D. (2007) Wheat value: improvements by feed technology, plant breeding and animal genetics. *World's Poultry Science Journal,* 63: 585-596

CROSS, D.E., MCDEVITT, R.M., HILLMAN, K. & ACAMOVIC, T. (2007) The effect of herbs and their associated essential oils on performance, dietary digestibility and gut microflora in chickens from 7 to 28 days of age. *British Poultry Science*, 48: 496-506.

ENGBERG, R.M., M.S. HEDEMANN, S. STEENFELDT & JENSEN, B.B. (2004) Influence of whole wheat and xylanase on broiler performance and microbial composition and activity in the digestive tract. *Poultry Science,* 83: 925-938.

FERKET, P. (2000) Feeding whole grains to poultry improves gut health. *Feedstuffs USA* (September), pp. 12-14.

HERNANDEZ, F., MADRID, J., GARCIA, V., ORENGO, J. & MEGIAS, M.D. (2004) Influence of two plant extracts on broiler performance, digestibility and digestive organ size. *Poultry Science*, 83: 169-174.

HUGHES, R.J. & CHOCT, M. (1999) Chemical and physical characteristics of grains related to variability in energy and amino acid availability in poultry, *Australian Journal of Agricultural Research,* 50: 689–702.

HUME, M.E., KUBENA, L.F. EDRINGTON, T.S. DONSKEKY, C.J. MOORE, R.W. RICKE S.C. & NISBET, D.J. (2003) Poultry digestive microflora biodiversity as indicated by denaturing gradient gel electrophoresis. *Poultry Science,* 82: 1100-1107.

INGLETT, G.E. (1974) *Wheat: Production and Utilisation*, The AVI Publishing Company, Inc., Westport, CT, USA.

JANG, I.S., KO, Y.H., KANG, S.Y. & LEE, C.Y. (2007) Effect of a commercial essential oil on growth performance, digestive enzyme activity and intestinal microflora population in broiler chickens. *Animal Feed Science and Technology*, 134: 304-315.

KAMEL, C. (2001) Tracing methods of action and roles of plant extracts in non-ruminants. In: Recent Advances in Animal Nutrition (eds.). Garnsworthy, P. C. and J. Wiseman, Nottingham University Press, Nothingham, UK.

LEE, K. W., H. EVERTS, H. J. KAPPERT, H. WOUTERSE, M. FREHNER & A. C. BEYNEN (2004) Cinnamanaldehyde, but not thymol, counteracts the carboxymethyl cellulose-induced growth depression in female broiler chickens. *International Journal of Poultry Science*, 3: 608- 612.

PÉRON, A., BENTO, M.H.L., SCHULZE, H., AMERAH, A.M. & JUIN, H. (2009) Effect of exogenous enzymes and essential oils on the performance of broiler chickens fed wheat-based diets. 17th European Symposium on Poultry Nutrition, Edinburgh, Scotland, pp 273.

PLAVNIK, I., MACOVSKY, B. & SKLAN, D. (2002) Effect of feeding whole wheat on performance of broiler chickens. *Animal Feed Science and Technology*, 96: 229-236.

PRESTON, C.M., MCCRACKEN, K.J. & MCALLISTER, A. (2000) Effect of diet form and enzyme supplementation on growth, efficiency and energy utilisation of wheat-based diets for broilers. *British Poultry Science*, 41: 324-331.

RAVINDRAN, V. HEW, L.I. RAVINDRAN, G. & BRYDEN, W.L. (2005) Apparent ileal digestibility of amino acids in feed ingredients for broiler chickens, *Animal Science,* 81: 85–97

RAVINDRAN, V., WU, Y.B., THOMAS, D.G. & MOREL, P.C.H. (2006) Influence of whole wheat feeding on the development of gastrointestinal tract and performance of broiler chickens. *Australian Journal of Agricultural Research*, 57: 21-26.

SAS (1997) SAS/STAT® User's Guide: Statistics. Version 6.12. SAS Institute Inc., Cary. NC.SHORT, F.J.P., GORTON, J., WISEMAN, J. & BOORMAN, K.N. (1996) Determination of titanium oxide added as an inert marker in chicken digestibility studies. *Animal Feed Science and Technology,* 59: 215–221.

SVIHUS, B. & HETLAND, H. (2001) Ileal starch digestibility in growing broiler chickens fed on a wheat-based diet is improved by mash feeding, dilution with cellulose or whole wheat inclusion. *British Poultry Science*, 42: 633-637.

SVIHUS, B., JUVIK, E., HETLAND, H., & KROGDAHL, A. (2004) Causes for improvement in nutritive value of broiler chicken diets with whole wheat instead of ground wheat. *British Poultry Science,* 45: 55-60.

TIIHONEN, K., KETTUNEN, H., BENTO, M.H.L., SAARINEN, M. LAHTINEN, S., OUWEHAND, A.C., SCHULZE, H. & RAUTONEN N. The effect of feeding essential oils on broiler performance and gut microbiota. *British Poultry Science*, **51:** 381-392.

WALLACE, R.J. (2004) Antimicrobial properties of plant secondary metabolites. *Proceedings of the Nutrition Society*, 63:621 – 629.

WU, Y.B. & RAVINDRAN V. (2004) Influence of whole-wheat inclusion and xylanase supplementation on the performance, digestive tract measurements and carcass characteristics of broiler chickens. *Animal Feed Science and Technology*, 116: 129-139.

WU, Y.B., RAVINDRAN, V., THOMAS, D.G., BIRTLES, M.J. & HENDRIKS, W.H. (2004) Influence of method of whole wheat inclusion and xylanase supplementation on the performance, apparent metabolisable energy, digestive tract measurements and gut morphology of broilers. *British Poultry Science*, 45: 385-394.

YANG, Y., IJI, P.A. & CHOCT, M. (2009) Dietary modulation of gut microflora in broiler

429 chickens: a review of the role of six kinds of alternatives to in-feed antibiotics. *World's* 430 *Poultry Science Journal,* 65: 97-114.

431 ZHANG, K.Y., YAN, F., KEEN, C.A. and WALDROUP, P.W. (2005) Evaluation of 432 microencapsulated essential oils and organic acids in diets for broiler chickens. *International* 433 *Journal of Poultry Science*, 4: 612-619.

**Table 1.** *Composition and calculated analysis (g/kg) of the basal diets*

| Ingredient as fed | Starter diet | Finisher diet |
|---|---|---|
| Wheat | 619.4 | 660.5 |
| Soybean meal 48 | 304.2 | 254.7 |
| Soybean oil | 34.98 | 46.03 |
| Dicalcium phosphate | 17.49 | 14.92 |
| Limestone | 1.166 | 0.964 |
| Lysine HCl | 2.41 | 1.56 |
| DL-Methionine | 2.54 | 2.04 |
| L-Threonine | 0.27 | 0.56 |
| Salt | 2.97 | 2.97 |
| Trace mineral-vitamin premix[1] | 3.00 | 3.00 |
| Titanium oxide | - | 3.00 |
| Xylanase[2] | + | + |
| *Calculated analysis* | | - |
| Metabolisable energy, MJ/kg | 12.55 | 12.97 |
| Crude protein | 220.0 | 200.0 |
| Lysine | 13.0 | 11.0 |
| Methionine + cystine | 9.50 | 8.50 |
| Calcium | 10.0 | 8.50 |
| Available phosphorus | 4.50 | 4.00 |

[1]Supplied per kilogram of diet: antioxidant, 100 mg; biotin, 0.2 mg; calcium pantothenate, 12.8 mg; cholecalciferol, 60 µg; cyanocobalamin, 0.017 mg; folic acid, 5.2 mg; menadione, 4 mg; niacin, 35 mg; pyridoxine, 10 mg; trans-retinol, 3.33 mg; riboflavin, 12 mg; thiamine, 3.0 mg; dl-α-tocopheryl acetate, 60 mg; choline chloride, 638 mg; Co, 0.3 mg; Cu, 3 mg; Fe, 25 mg; I, 1 mg; Mn, 125 mg; Mo, 0.5 mg; Se, 200 µg; Zn, 60 mg.

[2] Danisco Xylanase, Danisco Animal Nutrition, Marlborough, UK. The enzyme was included at a rate of 1kg/tonne. According to the manufacturer, this inclusion rate supplied 2000 xylanase units/kg feed.

**Table 2.** *Influence of wheat form and essential oil supplementation on the weight gain (g) feed intake (g) and feed per gain (g/g) of male broilers fed on wheat/soy-based diets*[1]

| | | 1-21 d | | | 22-35 d | | | 1-35 d | | |
|---|---|---|---|---|---|---|---|---|---|---|
| Wheat form | Essential oil | Weight gain | Feed intake | Feed per gain | Weight gain | Feed intake | Feed per gain | Weight gain | Feed intake | Feed per gain |
| Ground wheat | - | 939[b] | 1312 | 1.399 | 1406 | 2392 | 1.701 | 2344[c] | 3704 | 1.580 |
| | + | 1002[a] | 1355 | 1.366 | 1448 | 2385 | 1.647 | 2451[a] | 3740 | 1.540 |
| Whole wheat | - | 986[a] | 1310 | 1.328 | 1407 | 2356 | 1.675 | 2393[b] | 3666 | 1.531 |
| | + | 986[a] | 1325 | 1.344 | 1447 | 2400 | 1.659 | 2433[a] | 3725 | 1.531 |
| Pooled SEM | | 8.5 | 11.8 | 0.0137 | 10.5 | 33.9 | 0.0235 | 12.7 | 40.8 | 0.0143 |
| **Main effects** | | | | | | | | | | |
| *Wheat form* | | | | | | | | | | |
| Ground wheat | | 970 | 1333 | 1.382[a] | 1427 | 2388 | 1.674 | 2397 | 3722 | 1.560 |
| Whole wheat | | 986 | 1317 | 1.336[b] | 1427 | 2378 | 1.667 | 2413 | 3695 | 1.531 |
| *Essential oil* | | | | | | | | | | |
| - | | 962 | 1311[a] | 1.364 | 1407[a] | 2374 | 1.688 | 2369 | 3685 | 1.556 |
| + | | 994 | 1340[b] | 1.355 | 1447[b] | 2392 | 1.653 | 2442 | 3732 | 1.536 |
| *P* values | | | | | | | | | | |
| Wheat form | | 0.08 | NS | ** | NS | NS | NS | NS | NS | 0.06 |
| Essential oil | | *** | * | NS | *** | NS | NS | *** | NS | NS |
| Wheat form x essential oil | | *** | NS | 0.09 | NS | NS | NS | * | NS | NS |

[1] Each value represents the mean of 6 replicates (8 birds per replicate).

[a,b,c]Means in a column not sharing a common superscript are significantly different at *P* <0.05.

NS = not significant; * = *P*<0.05; ** = *P*<0.01; *** = *P*<0.0001.

**Table 3.** *Influence of wheat form and essential oil supplementation on ileal digestibility coefficients of nitrogen (IDCN) and gross energy (IDCGE), and apparent ileal digestible energy (AIDE, MJ/kg DM) of male broilers fed on wheat-soy diets (1-35 d)*[1]

| Wheat form | Essential oils | IDCN | IDCGE | AIDE |
|---|---|---|---|---|
| Ground wheat | - | 0.840 | 0.696 | 13.23 |
| | + | 0.861 | 0.738 | 14.02 |
| Whole wheat | - | 0.861 | 0.748 | 14.19 |
| | + | 0.865 | 0.736 | 13.96 |
| Pooled SEM | | 0.006 | 0.013 | 0.253 |
| **Main effects** | | | | |
| Wheat form | | | | |
| Ground wheat | | 0.850[a] | 0.717 | 13.62 |
| Whole wheat | | 0.863[b] | 0.741 | 14.08 |
| Essential oil | | | | |
| - | | 0.850[a] | 0.722 | 13.71 |
| + | | 0.863[b] | 0.737 | 13.99 |
| *P* values | | | | |
| Wheat form | | * | 0.08 | 0.09 |
| Essential oil | | * | NS | NS |
| Wheat form x essential oil | | NS | 0.06 | 0.06 |

[1] Each value represents the mean of 6 replicates (8 birds per replicate).

[a,b]Means in a column not sharing a common superscript are significantly different at $P < 0.05$.

NS = not significant; * = $P<0.05$.

**Table 4.** *Influence of wheat form and essential oil supplementation on gross morphology of the digestive tract of male broilers fedon wheat-soy diets (1-35 d)*[1]

| Wheat form | Ground wheat | | Whole wheat | | SEM | *P* values | | |
|---|---|---|---|---|---|---|---|---|
| Essential oil | - | + | - | + | | Wheat form (WF) | Essential oil (EO) | WF x EO |
| **Relative empty weights (g/kg BW)** | | | | | | | | |
| Pancreas | 1.67 | 1.63 | 1.68 | 1.75 | 0.059 | NS | NS | NS |
| Gizzard | 6.25[b] | 7.07[a] | 7.13[a] | 6.50[ab] | 0.223 | NS | NS | ** |
| Small intestine[2] | 16.89 | 15.90 | 16.60 | 16.80 | 0.420 | NS | NS | NS |
| Caeca | 0.985[ab] | 0.906[b] | 0.947[ab] | 1.031[a] | 0.038 | NS | NS | * |
| **Relative digesta content (g/kg BW)** | | | | | | | | |
| Gizzard | 1.57 | 2.10 | 1.94 | 2.61 | 0.389 | NS | NS | NS |
| Small intestine[2] | 33.56 | 34.51 | 31.69 | 31.60 | 1.70 | NS | NS | NS |
| Caeca | 1.635 | 1.594 | 1.621 | 1.624 | 0.204 | NS | NS | NS |
| **Relative length (cm/kg BW)** | | | | | | | | |
| Small intestine[2] | 88.00 | 80.36 | 83.12 | 84.01 | 2.10 | NS | NS | NS |
| Caeca | 8.66 | 8.44 | 8.78 | 8.70 | 0.215 | NS | NS | NS |

[a,b]Means in a row not sharing a common superscript are significantly different at $P < 0.05$.

[1]Each value represents the mean ± SEM of 12 birds.

[2]Small intestine = duodenum + jejunum + ileum.

**Table 5.** *Influence of wheat form and essential oil supplementation on overall species diversity*

| Feed Form | Essential oil | Band number | Shannon Weiner |
|---|---|---|---|
| Ground wheat | - | 13±0.6 | 1.03±0.17 |
| Ground wheat | + | 16±0.7[1] | 0.96±0.11 |
| Whole wheat | - | 14±0.5 | 0.73±018 |
| Whole wheat | + | 14±0.8 | 0.94±0.14 |

[1]Tended ($P$ = 0.07) to be different from unsupplemented ground wheat diet

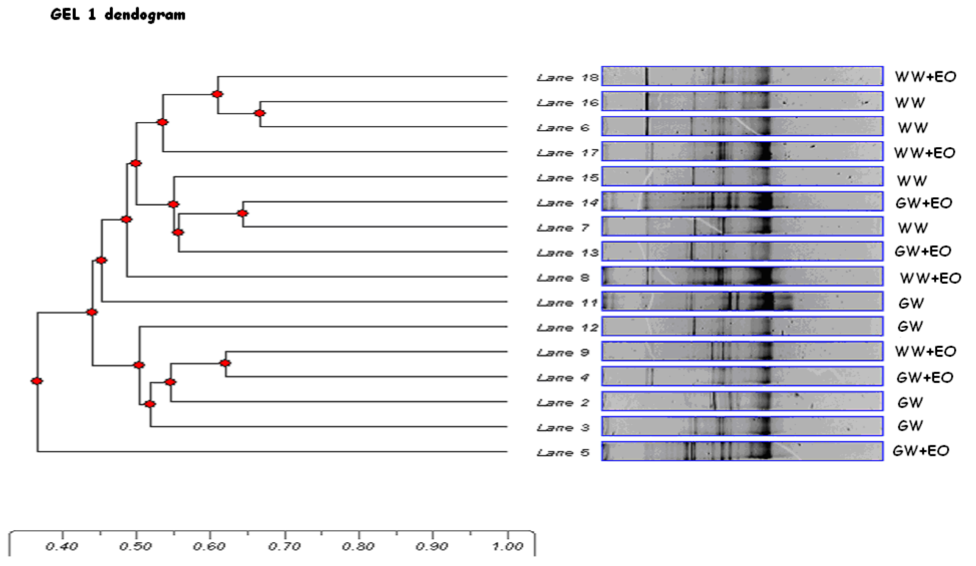



**Figure 1.** *Denaturant gradient gel electrophoresis profile (Gel 1) from ileal digesta of broilers as influenced by wheat form (whole wheat [WW] and ground wheat [GW]) and essential oils (EO). Relative similarity of band patterns of two denaturing gradient gel electrophoresis gels is indicated by their grouping on the dendogram and the percentage coefficient (bar).*

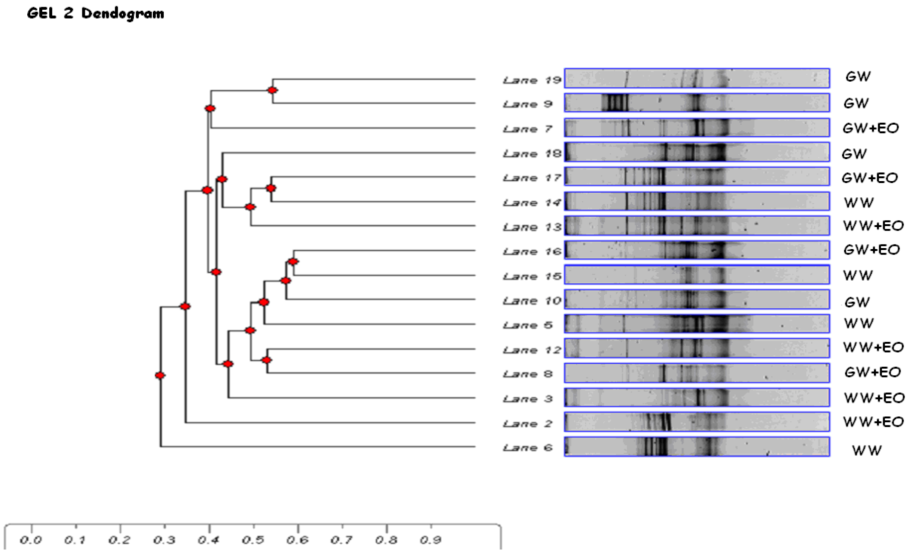



**Figure 2.** *Denaturant gradient gel electrophoresis profile (Gel 2) from ileal digesta of broilers as influenced by wheat form (whole wheat [WW] and ground wheat [GW]) and essential oils (EO). Relative similarity of band patterns of two denaturing gradient gel electrophoresis gels is indicated by their grouping on the dendogram and the percentage coefficient (bar).*

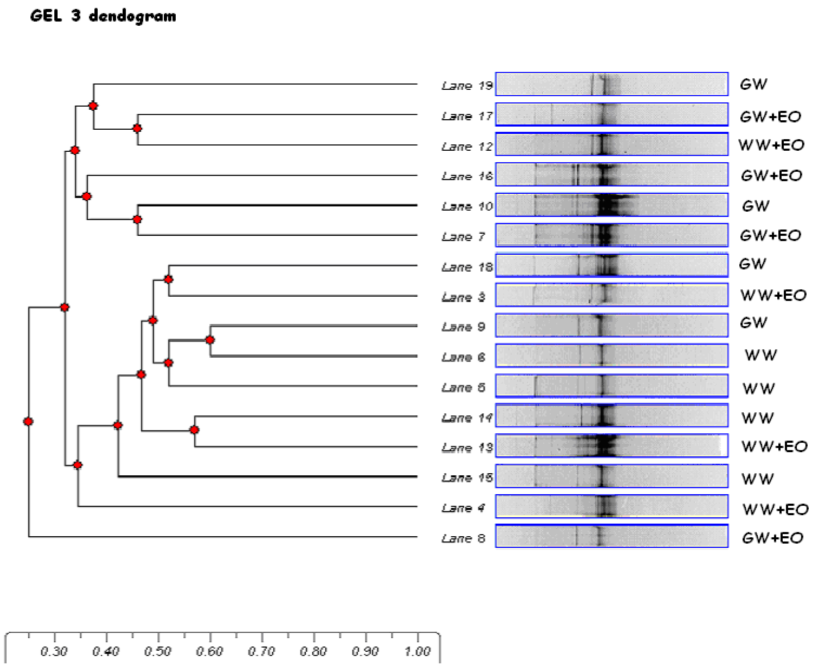



**Figure 3.** *Denaturant gradient gel electrophoresis profile (Gel 3) from ileal digesta of broilers as influenced by wheat form (whole wheat [WW] and ground wheat [GW]) and essential oils (EO). Relative similarity of band patterns of two denaturing gradient gel electrophoresis gels is indicated by their grouping on the dendogram and the percentage coefficient (bar).*