

Regulation of Intestinal Microflora and Productivity of Broiler Chickens by Prebiotic and Bioactive Plant Extracts

By

**Yatiana Vidana Arachchilage Janak Kamil
(Janak K. Vidanarachchi)**

B.Sc.Agric.(Hon), The University of Peradeniya, Peradeniya, Sri Lanka
M.Sc. The Memorial University of Newfoundland, St. John's, Newfoundland, Canada

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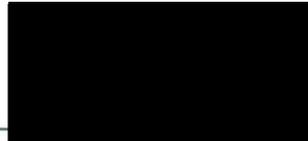
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DECLARATION

I certify that the substance of this thesis has not already been submitted for any degree and is not currently being submitted for any other degree or qualification.

I certify that any help received in preparing this thesis, and all sources used, have been acknowledged.

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Janak K. Yatiyana Vidana Arachchilage

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LIST OF ABBREVIATIONS

| | |
|----------------|--------------------------------------------------------------|
| AGPs | Antibiotic growth promoters |
| AME | Apparent metabolisable energy |
| ANOVA | Analysis of Variance |
| ARDRA | Amplified Ribosomal DNA Restriction analysis |
| BWG | Body weight gain |
| CCS | Counselling and Carrier Service |
| CE | Competitive exclusion |
| CFU | Colony forming unit |
| CP | Crude protein |
| <i>Cp</i> | <i>Clostridium perfringens</i> |
| CRC | Cooperative Research Centre |
| CSIRO | Commonwealth Scientific and Industrial Research Organisation |
| d | Day(s) |
| Da | Dalton |
| DM | Dry matter |
| DNA | Deoxyribose nucleic acid |
| DP | Degree of polymerisation |
| <i>E. coli</i> | <i>Escherichia coli</i> |
| ELISA | Enzyme-linked immunosorbent assay |
| EU | European Union |
| FCR | Feed conversion ratio |
| FI | Feed intake |
| GC | Gas chromatography |
| GIT | Gastrointestinal tract |
| h | Hour(s) |
| Ig | Immunoglobulin(s) |
| IU | International Units |
| kDa | Kilo Dalton |
| min | Minutes |
| MRS | De Man, Rogosa, Sharpe |
| MTPY | Modified tryptone-neutralized soy peptone-yeast extract |

| | |
|----------------|------------------------------------------------|
| MWCO | Molecular Weight Cut Off |
| NE | Necrotic enteritis |
| NRC | National Research Council |
| NSP | Non-starch polysaccharides |
| OD | Optical density |
| PBS | Phosphate buffered saline |
| PCR | Polymerase Chain Reaction |
| ppm | Parts per million |
| R _f | Ratio of fronts |
| RH | Relative humidity |
| RNA | Ribose nucleic acid |
| rRNA | Ribosomal RNA |
| RT | Room temperature |
| SCFA | Short chain fatty acid(s) |
| SEM | Standard error mean |
| spp. | Species |
| SRSA | School of Rural Science and Agriculture |
| TLC | Thin-layer chromatography |
| TPY | tryptone-neutralized soy peptone-yeast extract |
| UNE | University of New England |
| WSC | Water-soluble carbohydrate(s) |

LIST OF PUBLICATIONS

Janak K. Vidanarachchi, Lene L. Mikkelsen, Constantin Constantinoiu, Paul A. Iji and Mingan Choct. 2006. Plant extracts from Australian and New Zealand native plants as prebiotics in broiler chickens. 05-06 April 2006, Australian Veterinary Poultry Alliance Annual Meeting, Holiday Inn, Gold Coast, Australia. pp: 22-24.

Janak K. Vidanarachchi, Lene L. Mikkelsen, Ian Sims, Paul A. Iji and Mingan Choct. 2006. Selected plant extracts modulate the gut microflora in broiler. 19-22 February 2006, Australian Poultry Science Symposium, University of Sydney, Australia. Vol. 18, pp:145-148.

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Janak K. Vidanarachchi, Lene L. Mikkelsen, Ian Sims, Paul A. Iji and Mingan Choct. 2005. Phytobiotics: Alternatives to antibiotic growth promoters in monogastric animal feed. In *Recent Advances in Animal Nutrition in Australia*. Ed. P. Cronje. Vol. 15, pp:131-144.

OUT LINE OF THE THESIS

This thesis presents a review of the appropriate literature and results from the experiments that were conducted with water-soluble carbohydrate extracts from four Australian and New Zealand plants, *Arthropodium cirratum* (Rengarenga lily), *Cordyline australis* (Cabbage tree), *Acacia pycnantha* (*Acacia* or Golden wattle) and the seaweed, *Undaria pinnatifida* (*Undaria* spp. or Wakame). Plant extracts and commercially available prebiotic compounds, with a chemical composition of which was similar to that of the plant extracts were examined for their use as alternatives to antibiotic growth promoters (AGPs) in broiler chickens.

Chapter 1 briefly describes the background information and justifies the importance of research in the topic of interest, leading to the major hypothesis and objectives for conducting experimental studies. Chapter 2 is a review of literature on gut microflora in poultry, commonly used AGPs, their modes of action and alternatives to AGPs in poultry feed. Special emphasis is given to prebiotics and phytobiotics as alternatives for AGPs in poultry and other monogastric animals and their possible modes of action are discussed in detail.

The isolation and characterisation of water-soluble prebiotic compounds from the plant extracts [*Arthropodium cirratum* (Rengarenga lily), *Cordyline australis* (Cabbage tree), *Acacia pycnantha* (*Acacia* or Golden wattle) and the seaweed *Undaria pinnatifida* (*Undaria* or Wakame)] are reported in Chapter 3. Qualitative and quantitative analytical techniques and measurements were carried out in order to characterise the chemical composition of plant extracts.

Chapters 4 and 5 describe the results from two *in vivo* experiments that were designed to test the effects of plant extracts on performance, organ development, gut morphology, microbial populations and microbial activity in broiler chickens. In both these trials a negative control (without any supplements) and a positive control (45 mg Zn-bacitracin/kg) were used for comparative purpose. In Chapter 4, the effects of Rengarenga lily extract and commercially available fructan compound (Frutafit) at two levels (5 g/kg and 10 g/kg) on digestive organ development, gut morphology, nutrient digestibility and growth performance of broiler

chickens are discussed. Chapter 5 reports the effects of dietary plant extracts (Cabbage tree extract, *Undaria* seaweed extract, and *Acacia* extract) at two levels (5 g/kg and 10 g/kg) on ileal and caecal gut microflora composition, microbial fermentation activity, as well as their effects on gut morphology and growth performance in broiler chickens.

The molecular and biochemical results characterising lactobacilli isolated from ileal and caecal digesta of broilers fed prebiotic plant extracts are described in Chapter 6. Molecular characterisation of lactobacilli was carried out using the Amplified Ribosomal DNA Restriction Analysis (ARDRA) and partial 16S rRNA gene sequencing. The Ph-48 generalised PhenePlate system was used for evaluation of fermentation characteristics of the isolated lactobacilli.

In Chapter 7 the effects of two plant extracts (*Acacia* extract and Rengarenga lily extract) and two commercially available prebiotic compounds (Fibregum and Raftifeed) and Zn-bacitracin on gut microflora composition and prevention of necrotic enteritis (NE) outbreak in birds challenged with *Clostridium perfringens* (*Cp*) are presented. In addition, the effects of dietary supplements on humoral immune responses and gut morphology were also investigated during pre- and post-infection period.

Finally, the implications and significance of the major findings and an overview of effects of the studied plant extracts and prebiotic compounds, as alternatives for AGPs, on growth performance and health of broiler chickens are discussed. The major highlights of each experiment are presented in the relevant chapter in form of an abstract.