



# Final Report

Project code: 19 - 101

Prepared by: Dr Amy Moss

Date: 30/07/2020

Review of the importance of sampling and  
sampling methodology in poultry production

Milestone 4

© 2020 Poultry Hub Australia All rights reserved.

## Review of the importance of sampling and sampling methodology in poultry production

The information contained in this publication is intended for general use to assist public knowledge and discussion and to help improve the development of sustainable industries. The information should not be relied upon for the purpose of a particular matter. Specialist and/or appropriate legal advice should be obtained before any action or decision is taken on the basis of any material in this document. Poultry Hub Australia, the authors or contributors do not assume liability of any kind whatsoever resulting from any person's use or reliance upon the content of this document. This publication is copyright. However, Poultry Hub Australia encourages wide dissemination of its research, providing the Hub is clearly acknowledged. For any other enquiries concerning reproduction, contact the Poultry Hub Office on 02 6773 1855.

This project is supported by Poultry Hub Australia through funding from AgriFutures Australia as part of its AgriFutures Chicken Meat Program.



### **Researcher Contact Details**

Dr Amy Moss  
University of New England  
0490147735  
Amoss22@une.edu.au

In submitting this report, the researcher has agreed to Poultry Hub Australia publishing this material in an edited form.

### **Poultry Hub Australia Contact Details**

Poultry Hub Australia  
CJ Hawkins Homestead, Ring Road  
University of New England  
Armidale NSW 2350  
02 6773 1855  
[poultryhub@une.edu.au](mailto:poultryhub@une.edu.au)  
[www.poultryhub.org](http://www.poultryhub.org)

## Project Summary

<b>Project Title</b>	Review of the importance of sampling and sampling methodology in poultry production
<b>Project No.</b>	19 - 101
<b>Date</b>	Start: 01/01/20      End: 01/08/20
<b>Project Leader(s)</b>	Dr Amy Moss
<b>Organisation</b>	University of New England
<b>Email</b>	amos22@une.edu.au
<b>Project Aim</b>	The aims of this review were to; i) model the extent that variation in protein in feed ingredients affects expected performance and profits for the Australian poultry industry; and ii), review the existing literature on sampling methodology and present options the industry may take to improve the accuracy of feed formulation.
<b>Background</b>	Accurate feed formulation is vital to ensure poultry are receiving an optimal diet and nutrients are not in under- or over-supply. However, this is difficult when the nutrient specifications of feed ingredients are highly variable. In order to help reduce this variability, appropriate sampling methodology is critical. Nevertheless, recommended methodology and depth of detail within technical articles varies greatly. Some 45 years ago Lerman and Bie (1975) concluded that improper sampling technique is a major component of ingredient variability; nevertheless, few animal nutrition studies report the sampling technique used, nor is the potential economic cost often discussed.
<b>Research Outcome</b>	The extent that variation of protein content in feed ingredients affects expected performance and profits for the Australian poultry industry was modelled (EFG Broiler Model). Within withdrawal diets alone (formulated to 19.2 g/kg crude protein; CP), there is approximately a one in 10 chance that diets will fall below 182 g/kg CP, and diets may fall as low as 162 g/kg CP; which was modelled to lower the gross margin from \$21.26/m <sup>2</sup> (\$1.417/bird/cycle) to 7.88/m <sup>2</sup> (\$0.525/bird/cycle) – a reduction in profits of 63%. Therefore, it is possible to incur a difference of up to \$26,753 in gross margin from one cycle of 30,000 broilers by simply overestimating the nutrient content of feedstuffs.
<b>Impacts and Outcomes</b>	This review highlighted that improving the understanding and implementation of proper sampling methodology is of great importance for the Australian poultry industry. Nevertheless, sampling methods within the literature were limited and varied. As losses may be substantial, sampling systems and variability within ingredients should be a priority research theme for the poultry industry.
<b>Publications</b>	Moss AF, Chrystal PV, Crowley TM, Pesti GM (2020) Variability in nutrient measurement of feed ingredients has consequences for profitability in the Australian poultry industry. Submitted to JAPR.

## Project Status

Have the aims of the project been achieved?	Yes
Date final report was due	01/08/2020
Have any publications been released during this project?	No
Are there publications that are planned/in preparation that will be release after the completion of this project?	Yes
Has any IP arisen from this project?	No
Is there any reason to embargo this final report?	No

## Executive Summary

Feed accounts for more than 65% of live production costs of poultry production, thus, accurate feed formulation is vital to ensure poultry are receiving an optimal diet and nutrients are not in under- or over-supply. However, this is difficult when the nutrient specifications of feed ingredients are highly variable. In order to help reduce this variability, appropriate sampling methodology is critical. It is often understood that increased variability in ingredients due to poor sampling technique is detrimental to industry, but the potential economic cost of poor sampling is often not appreciated.

Therefore, the extent that variation in protein in feed ingredients affects expected performance and profits for the Australian poultry industry was modelled. Protein is an expensive and crucial macronutrient component of poultry diets; thus, the model focussed on the extent that variation in protein in feed ingredients affects expected performance and profits for the Australian poultry industry. The variability (coefficient of variation; CV) in crude protein of the components of Australian poultry diets were estimated from Moss (2020), and simulations were performed to estimate the likelihood a diet would fall below recommendations using Excel 2016, NORMINV function (10,000 simulations/diet). All prices are in \$AUD. CV's worsened in finisher and withdrawal diets as more canola meal is generally incorporated to meet the increasing energy requirement, but wheat was the single greatest source of variability in crude protein content of diets. Within withdrawal diets formulated to 19.2 g/kg crude protein from book values, there is approximately a 10% probability (or one in 10 diets) that it will fall below 182 g/kg CP, and diets may fall as low as 162 g/kg CP; which was modelled to lower the gross margin from \$21.26/m<sup>2</sup> (\$1.417/bird/cycle) to 7.88/m<sup>2</sup> (\$0.525/bird/cycle) – a reduction in profits of 63%. Hence, it is possible to incur a difference of up to \$26,753 in gross margin from one cycle of 30,000 broilers by simply overestimating the nutrient content of feedstuffs. Assuming a poultry company may produce approximately 1000 broiler cycles per year, this equates to a loss of up to \$26 million.

Following the determination of the potential cost of poor sampling and the high variability in Australian feed ingredients, a review of sampling methodology literature was conducted. Grab samples are commonly employed within industry to sub-sample for its ease, however it is reported to generate one of the largest standard deviations and worst representativeness of 17 methods tested (Petersen et al., 2004). Recommended methodology and depth of detail within technical articles varies greatly and doesn't always reflect the recommendations of the AOAC; a non-profit scientific association that publishes standardised analytical methods.

Additionally, there are limited Australian government sampling recommendations. As losses may be substantial, sampling systems and variability within ingredients should be a priority research theme for the poultry industry and likely also for many other intensive animal production systems within Australia, as the challenges described in this paper are met across many industries.

## Table of Contents

Introduction	7
Objectives	8
Methodology	9
Discussion of Results	10
Implications	11
Recommendations	11
Acknowledgements	12
Media and Publications	12
Intellectual Property Arising	12
References	12
Appendices	14

## Introduction

Feed accounts for more than 65% of live production costs of poultry production (Wilkinson, 2018); thus, accurate feed formulation is vital to ensure poultry are receiving an optimal diet and nutrients are not in under- or over-supply. However, this is difficult when the nutrient specifications of feed ingredients are highly variable (Moss et al., 2020). Within industry, chemical analyses of feed ingredient samples are impractical due to the cost and time involved, with as much as 3 million tonnes of feed pelleted for the Australian integrated broiler industry per annum (ACMF 2020).

Consequently, near-infra red (NIR) calibrations are often used within integrated operations to instantaneously estimate the nutrient composition of feedstuffs in order to keep up with demand. However, NIR calibrations are only as accurate and representative of the feedstuff as the sample that was taken. This has been an ongoing issue within industry for some time. Lerman and Bie (1975) published a review describing the substantial variation of nutrient composition in feed ingredients – grains and protein meals in particular – and modelled the potential economic cost of this uncertainty. It was concluded that improper sampling technique is a major component of this variability and correct sampling is vital to ensure the accuracy of diet composition and optimal animal production. Nevertheless, some 45 years later, few animal nutrition studies report the sampling technique used or the variation caused by inappropriate sampling (Jones et al., 2018). Ingredient variation on feed formulation costs and bird responses have been identified in recent years (Jurgens et al., 2012), but they have not been practically applied in a framework that is useful to producers, nor do they indicate the modern-day economic cost of such uncertainty. Thus, industry still faces the challenge of how to account for this variation in feed formulation (Kleyn et al., 2013).

Additionally, recommended sampling methodology and depth of detail within technical articles varies greatly, meaning that finding appropriate sampling methodology may be difficult. This is compounded by the fact that few research papers report the sampling methodology used.

Protein is an expensive and crucial macronutrient component of poultry diets, but it can be sampled and tested relatively easily compared to other nutrients, such as starch or fat. Therefore, this review will firstly model the extent that variation in protein in feed ingredients affects expected performance and profits for the Australian poultry industry; and secondly, review the existing literature on sampling methodologies and present options the industry may take to improve the accuracy of feed formulation.

## Objectives

The aims of this review were to;

- i) model the extent that variation in protein in feed ingredients affects expected performance and profits for the Australian poultry industry; and,
- ii) review the existing literature on sampling methodology and present options the industry may take to improve the accuracy of feed formulation.

It was anticipated that the modelling exercise and review demonstrating the importance of accurate sampling methodology and comparing the available methodologies will improve the adoption and development of accurate sampling practices for Australian poultry industry and researchers. Thus allowing poultry nutritionists to achieve more precise diet formulation and realise improvements in production efficiency, reduced safety margins and feed costs, while also improving the accuracy of Australian research.

## Methodology

### *Modelling the impact of sampling and variability of nutrients on poultry production*

Starter, grower, finisher and withdrawal diets used in the following exercise were formulated to most accurately represent standard modern Australian broiler diets (Table 2) using EFG Broiler Model software (EFG Software, 2020). Once the standard deviation and mean of a dietary component is known, assuming normality, simulations can be performed to estimate the likelihood a diet mixed to optimal specifications may in fact fall below recommendations. This was performed for the following example using Excel 2016, NORMINV function, 10,000 individual simulations per diet.

In order to simulate the economic cost, the median, highest and lowest dietary crude protein levels possible identified by the Excel simulation for the starter, grower, finisher and withdrawal diets were modelled using EFG Broiler Model software (EFG Software, 2020). To formulate the extreme diets, the feed ingredient crude protein level as well as the diet nutrient specification were adjusted to give the desired extreme low or high dietary crude protein level with essentially the same proportions of feed ingredients and diet costs. The simulation was based on Ross 308 genetics (2019), set to 30,000 birds per cycle, placed at an initial stocking density of 15 birds/m<sup>2</sup> with estimated variable costs (chicks, vaccination, catching, cleaning, processing, etc.,) totalling 230 cents/bird/cycle (all prices in \$AUD) and fixed costs (labour, insurance, repairs, etc.,) totalling \$40/m<sup>2</sup>/year. Down time between cycles was set to 10 days and estimated flock mortalities at 5% to 42 days post-hatch. Environmental conditions were set to the Ross 308 guidelines and two cropping cycles were set over the total 42 day grow-out period. Estimated sales were set at 30% sold dressed (\$4.50 dressed weight and \$3.80 downgraded) and 70% sold processed (breast \$7.50, thigh \$4.70, drum \$4.00, wing \$4.90).

### *Review of sampling methodology literature*



There are very few research papers which identify or demonstrate the importance of proper sampling technique for poultry research conclusions and sustainable poultry industry outcomes (databases searched include Google Scholar, Web of Science, CAB direct and PubChem using the search terms “feed sampling procedures” and “poultry”). Additionally, Australian IP was searched using the Australian government IP search engine, AusPat (<http://pericles.ipaustralia.gov.au/ols/auspat/>) and searched internationally using the World Intellectual Property Organisation PATENTSCOPE (<https://patentscope.wipo.int/search/en/search.jsf>). Both searches were performed using the terms “poultry feed sampling procedures” and no IP for feed sampling procedures in poultry was found.

However, there are technical documents available (found via Google, search terms “feed sampling procedures” and “poultry”), which were discussed within the review. Included in these technical articles was industry specific sampling methodology and broader Australian Government Sampling Regulations/Recommendations.

## Discussion of Results

### *Modelling the impact of sampling and variability of nutrients on poultry production*

Simulations were performed for starter, grower, finisher and withdrawal diets to estimate the likelihood a diet mixed to optimal specifications may in fact fall below recommendations. Within withdrawal diets formulated to 19.2 g/kg crude protein from book values, there is approximately a 10% probability (or one in 10 diets) that it will fall below 182 g/kg CP. Given that poultry feed accounts for 65% of total production cost, how much could poor sampling technique and high ingredient variability be costing the Australian poultry industry?

Gross profit is best measured as a margin per unit of area over time. In the current example, all birds were grown to a simulated fixed cycle of 42 days post-hatch with a 10 day down-time, resulting in 7.019 cycles (or placements) per year. Thus a comparison of margin/m<sup>2</sup> of shed (or barn) floor space between the various simulations has been used since all time periods in this instance are equal. However, if the target response per broiler is based on a set live weight, then variable cycles ensue and time periods become relevant. The EFG broiler growth simulation using the median protein values returned the greatest financial gross margin of \$21.26/m<sup>2</sup> whilst the return on the minimum dietary protein was 63% lower (7.88/m<sup>2</sup>) and the maximum dietary protein 21% lower (\$16.88/m<sup>2</sup>) (Table 1). Thus, it is possible to incur a difference of up to \$26,753 in gross margin from one cycle of 30,000 broilers by simply overestimating the nutrient content of feedstuffs. Therefore, sampling error has the possibility to generate large financial consequences, with the overestimation of the nutrient content of feed ingredients (ie: feed ingredients being lower in nutrient content than their perceived value) representing the largest potential cost.

It is also important to also note that while the highest calculated CP level had less of an impact to profits than the low CP level, it may have a larger environmental impact. Nitrogen Excretion was highest on day 42 under the high CP diet at 4329 mg/bird/day than the medium (3293 mg/bird/day) or low (2492 mg/bird/day) CP diets. Thus, while the cost

impact may not be as great in the high CP diet than the low CP diet, environmental impacts are of a greater extent.

### *Review of sampling methodology literature*

Technical bulletins describing sampling procedures for poultry feed are available (Herrman, 2001; AAFCO, 2014; Malomo and Ihegwuagu, 2017; FAO, 2008; Meehan and Sedivec, 2018; U.S. Food and Drug Administration, 2019); however, recommended sampling methodology and depth of detail varies greatly. Additionally, the technical bulletins describing sampling techniques (Herrman, 2001, AAFCO, 2014, Meehan and Sedivec, 2018) discuss methods to get a more accurate sample from a hand or bag probe. However, AOAC international has identified stream sampling as a more effective procedure compared to probe sampling, whereby small portions are sampled from the stream at periodic intervals and the portions are combined into a large aggregate sample, which can be done effectively with an automatic cross-cut sampler (Davis et al., 1980). This obviously will only be effective for feedstuffs which flow, such as grain. For sampling methodology to be employed it must be practical within an industry setting. For example, within a feed mill, probe samples may be quickly obtained from trucks full of grain as they arrive to the mill to determine if the grain is appropriate to accept. Taking a stream sample at this point is not practical as the grain would need to be unloaded from the truck. However, upon loading the accepted grain into a silo, there may be opportunity to collect more accurate stream samples to more accurately assess the grain's nutrient content for the purpose of feed formulation. While multiple truckloads of grain are often contained within a silo, the data may be aggregated to attain a more accurate approximation of the average nutrient content.

Another important consideration is that the primary sample taken must also be of substantial size and then reduced via material reduction and sub-sampling techniques to achieve the degree of representivity required (Petersen et al., 2004). Some guidelines of the size of samples to take from various feedstuffs are provided in Malomo and Ihegwuagu (2017). Grab samples are commonly employed within industry to sub-sample for its ease, however it was reported to generate one of the largest standard deviations and worst representativeness of 17 methods tested in Petersen et al. (2004). In contrast, rolling dividers such as the Boerner Divider were recommended (Herrman, 2001; Petersen et al., 2004) as they divided samples with the greatest accuracy to attain a sample small enough with which to perform analysis.

The Official Journal of the European Union states that methods used for sampling should comply with Union rules and provides a comprehensive guide to sample preparation of animal feed stuffs (International Organization for Standardization, 2012) which could prove useful outside the EU; however, the method is not provided open-access and thus there are barriers to its use. Nevertheless, an EU guide describing sampling and mixing techniques and equipment to sample feedstuffs for GMO analysis is available open-access and describes many of the acceptable sampling techniques for animal feeds (European Union, 2014).

Grain Trade Australia provides a fact sheet on appropriate sampling equipment and some procedures for static grain sampling from road trucks (Grain Trade Australia, 2018). However, it is identified within this document that the research defining their recommendations “was conducted many years ago”, that “studies indicate variability among

probe types” and due to the variability in probe type, depth of the load and commodity type, obtaining a representative sampling via their methodology is not always possible. Furthermore, the procedures outlined in this document are likely not applicable to small scale poultry research facilities. These methods are only for grain feed ingredient samples and do not cover protein meals or pelleted feeds. Within the document, it is stated that “as there has not been any data provided on the financial loss to industry of inappropriate sampling systems, this research to date has not been considered a high priority”. However, as shown above the losses are likely substantial and thus sampling systems should be a high priority research theme.

## Implications

Within the Grain Trade Australia fact sheet (Grain Trade Australia, 2018), it is stated that “as there has not been any data provided on the financial loss to industry of inappropriate sampling systems, this research to date has not been considered a high priority”. However, losses are likely substantial – as demonstrated within the modelling exercise, it is possible to incur losses of up to \$26,000 in profits from one cycle of 30,000 broilers, and potentially this may cost the Australian chicken-meat industry up to a total of 696 million in lost profits due to the variability of Australian feed ingredients. Assuming a poultry company may produce approximately 1000 broiler cycles per year, this equates to a loss of up to \$26 million. Thus, sampling systems should be a high priority research theme for the poultry industry and also for many other intensive animal production systems within Australia, as the challenges described in this paper exist within many agricultural industries.

## Recommendations

Misestimating the nutrient content of feed ingredients clearly has the potential to have vast economic consequences for the poultry industry. Thus, improving sampling methods and access of industry and researchers to clear information about sampling techniques and proper reporting is a key priority. There is potentially large economic consequences arising from poor sampling methodology and the variability within feed ingredients. Therefore, the effect of variation in feed ingredients on performance and profits for industry nutritionists is of great importance, and it is hoped that this review has highlighted this underestimated issue.

Nevertheless, proper sampling methods provided within the literature provide a multitude of differing recommendations, and there are limited Australian government sampling recommendations. Additionally, grab sampling is commonly employed within industry to sub-sample for its ease, however it is reported to generate one of the largest standard deviations and worst representativeness of 17 methods tested (Petersen et al., 2004). As losses may be substantial, sampling systems and variability within ingredients should be a priority research theme for the poultry industry and likely also for many other intensive animal production systems within Australia, as the challenges described in this paper are met across many industries. An extension project to develop an infographic on the importance of sampling and best methods to use could be a good option to improve awareness throughout industry and be sent out to producers and feed mills.

Finally, other approaches to help mitigate this risk include the improvement of descriptive data that is provided in book values (e.g.: standard deviation and normality of the distribution), adoption of NIR technologies where possible, and the implementation of feed formulation strategies (such as stochastic feed formulation) to minimise the impact of variability within ingredients.

## Acknowledgments

I would like to acknowledge and thank Poultry Hub Australia for funding this project and for their guidance, encouragement and support. I would also like to thank Mr Peter Chrystal and Professor Gene Pesti for their guidance and contributions throughout this project.

## Media and Publications

Moss AF, Chrystal PV, Crowley TM, Pesti GM (2020) Variability in nutrient measurement of feed ingredients has consequences for profitability in the Australian poultry industry. Submitted to JAPR.

Moss AF, Chrystal PV, Crowley TM, Pesti GM (2020) Variability in nutrient measurement of feed ingredients compromises profitability in the Australian poultry industry. In preparation for submission to the Australian Poultry Science Symposium.

## Intellectual Property Arising

No IP has arisen from this project.

## References

Davis ND, Dickens JW, Freie RL, Hamilton PB, Shotwell OL, Wyllie TD, Fulkerson JF. Protocols for surveys, sampling, post-collection handling, and analysis of grain samples involved in mycotoxin problems. *J Assoc Off Anal Chem* 1980; 63:95-102.

EFG Software. Broiler Growth Model. 2020. <http://www.efgsoftware.net/poultry-programs/broiler-growth-model> [Accessed 20/04/2020].

European Union. Guidelines for sample preparation procedures in GMO analysis. Joint Research Centre, European Union. 2014. <https://publications.jrc.ec.europa.eu/repository/bitstream/JRC94042/lbna27021enn.pdf> [Accessed 12/05/2020].

FAO. Code of Practice on Good Animal Feeding, Section 6. 2008. <http://www.fao.org/3/i1379e/i1379e06.pdf> [Accessed 20/11/2019].

- Grain Trade Australia Technical Guideline Documents. 2018. <http://www.graintrade.org.au/grain-industry-code-practice/gta-technical-guidelines> [Accessed 24/06/2019].
- Herrman T. MF-2036 Feed Manufacturing, Sampling: Procedures for Feed. Kansas State University. 2001. <https://www.bookstore.ksre.ksu.edu/pubs/mf2036.pdf> [Accessed 20/11/2019].
- International Organization for Standardization. Animal feeding stuffs — Guidelines for sample preparation; ISO 6498:2012. 2012. <https://www.iso.org/standard/52285.html> [Accessed 12/05/2020].
- Jones AM, Woodworth JC, Vahl CI, Tokach MD, Goodband RD, DeRouchey JM, Dritz SS. Assessment of sampling technique from feeders for copper, zinc, calcium, and phosphorous analysis. *J Anim Sci* 2018; 96:4611-4617.
- Jurgens M, Bregendahl K, Coverdale J, Hansen S. Animal feeding and nutrition. Eleventh ed. Kendall Hunt publishing Company, Iowa, United States of America, 2012.
- Kleyn R. Chicken nutrition. A Guide for Nutritionists and Poultry Professionals. British library press, Leicestershire, London, UK, 2013.
- Lerman PM, Bie SW. Problems in determining the best levels of essential nutrients in feedingstuffs. *J Agric Sci* 1975; 84:459-468.
- Malomo GA, Ihegwuagu NE. Some Aspects of Animal Feed Sampling and Analysis. 2017. <https://cdn.intechopen.com/pdfs/57363.pdf> [Accessed 20/11/2019].
- Meehan M, Sedivec K. AS1064 Sampling Feed for Analysis, North Dakota State University. 2018. <https://www.ag.ndsu.edu/publications/livestock/sampling-feed-for-analysis> [Accessed 04/12/2019].
- Moss AF, Crowley TM, Choct M. Compilation and assessment of the variability of nutrient specifications for commonly used Australian feed ingredients. *Proc, Aust Poult Sci Symp* 2020; 31:52.
- Petersen L, Dahl CK, Esbensen KH. Representative mass reduction in sampling—a critical survey of techniques and hardware. *Chemom Intell Lab Syst* 2004; 74:95-114.
- U.S. Food and Drug Administration. Investigations operations manual, Chapter 4, Sampling. 2019. <https://www.fda.gov/media/75243/download> [Accessed 20/11/2019].
- Wilkinson S. Big data for poultry—what is possible? *Proc, Aust Poult Sci Symp* 2018; 29:152.

## Appendices

**Table 1** EFG model simulation of economic analysis per batch (cycle) of broilers (total 30000), placed at 15 broilers/m<sup>2</sup> and reared to 42 days post-hatch in 2000 m<sup>2</sup> floor-space sheds (or barns).

Simulation	Gross margin in AU\$					
	Per bird		Per kilogram		Per unit area (m <sup>2</sup> )	
Crude protein level	Per cycle	Per year	Per cycle	Per year	Per cycle	Per year
Minimum	0.525	3.689	0.060	0.423	7.882	55.33
Median	1.417	9.948	0.133	0.934	21.26	149.22
Maximum	1.125	7.897	0.112	0.786	16.87	118.45
Maximum difference	0.892	6.259	0.073	0.511	13.38	93.89