Final report **summary**

Branched-chain amino acids in wheat-based, crude protein-reduced diets

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Abstract

The successful development of a reduced-protein broiler diet is an ongoing focus for the Australian chicken meat industry. Reduced-CP diets have the potential to halve the industry's dependence on imported soybean meal, and thus reduce its exposure to fluctuations in global soybean meal prices. Such diets can also reduce the carbon footprint of feed production, improve litter quality, reduce nitrogen pollution and ammonia emissions, and enhance bird welfare.

However, several tangible obstacles are thwarting the development of reduced-CP diets. Broiler diets are formulated to meet ideal (digestible) amino acid ratios (IAAR), but the likelihood is that the IAAR for a reduced-CP diet is not identical to that for a standard diet. Thus, because of their nutritional importance, there is a need to accurately identify the branched-chain amino acid (BCAA) requirements for broiler chickens offered reduced-CP diets.

This project evaluated elevated inclusions of BCAAs in wheat- and sorghum-based diets, and L-carnitine inclusions in sorghum-based diets with three tiers of crude protein (CP) concentrations. Elevated BCAA inclusions in wheat-based diets did not influence growth performance in feeding study BCAA 01. However, elevated BCAA inclusions significantly enhanced growth performance in broilers offered sorghum-based diets in BCAA 02, but depressed growth performance when included in wheat-based diets. In BCAA 03, L-carnitine inclusions significantly improved weight gain and feed conversion ratio (FCR) in broilers offered 160 g/kg CP sorghum-based diets, but did not influence the performance of broilers offered 190 and 220 g/kg CP diets.

The selection of a feed grain for a reduced-CP diet is pivotal because starch digestion rates, protein contents and amino acid profiles assume importance. Consideration is given to the likelihood that non-bound amino acids are more prone to be deaminated, resulting in excessive ammonia levels, which require detoxification.

Background

Australia imported 1.18 million tonnes of soybean meal in 2020 (IndexMundi), mainly from Argentina, at an approximate landed cost of $750 per tonne. The bulk of these shipments, perhaps more than 700,000 tonnes, was absorbed by the domestic chicken meat industry. Consequently, the sustainability of this industry is highly vulnerable to fluctuations in global soybean meal prices, which are likely to escalate.

The successful development of reduced-CP diets for broiler chickens has the potential to halve this soybean meal dependence. Axiomatically, reduced-CP diets contain less soybean meal because it is partially replaced by an array of non-bound (synthetic, crystalline) amino acids. Indeed, non-bound amino acids are used as alternatives to soybean meal in chicken meat production (Selle et al., 2020a). Moreover, as reviewed by Greenhalgh et al. (2020), there are additional compelling reasons to develop reduced-CP broiler diets. These include less nitrogen pollution and fewer ammonia emissions, better litter quality, lower incidences of foot pad lesions, enhanced bird welfare, and attenuated populations of potentially pathogenic microbiota in the gut.

Nevertheless, several tangible obstacles are thwarting the development of reduced-CP diets, as outlined by Liu et al. (2021). Broiler diets are formulated to meet ideal (digestible) amino acid ratios (IAAR), but the likelihood is that the IAAR for a reduced-CP diet is not identical to that for a standard diet, as demonstrated by Macelline et al. (2021a). Thus, because of their nutritional importance, there is a need to accurately identify the BCAA requirements for broiler chickens offered reduced-CP diets.

Objectives

The original objective of this project was to evaluate elevated inclusions of the BCAAs leucine, isoleucine and valine in wheat-based, CP-reduced broiler diets. All three BCAAs are 'essential' – leucine has anabolic effects as it can promote protein synthesis and either isoleucine or valine is probably the fourth limiting amino acid in broiler diets.

However, antagonisms between BCAAs are established (Calvert et al., 1982). Curiously, recommended dietary leucine levels are higher for young pigs (132) than broiler chickens (109), relative to lysine (100), although the leucine fraction of whole-body protein is identical in the two animal species (Wu, 2014). Moreover, positive responses to dietary inclusions of BCAAs have been recorded in pigs (Zheng et al., 2016, 2017). Thus, the hypothesis was that broilers would be advantaged by elevated leucine inclusions in diets provided there were concomitant increases in isoleucine and valine to accommodate BCAA antagonisms.

Research

The three feeding studies of this project (BCAA 01, 02, 03) determined key parameters in broiler chickens in response to several dietary treatments. These parameters included growth performance, relative abdominal fat pad weight, nutrient utilisation, jejunal and ileal apparent digestibility coefficients, disappearance rates (g/bird/day) of starch, protein and amino acids, starch:protein disappearance rate ratio, and free amino acid plasma concentration.

> "The successful development of reduced-CP diets for broiler chickens has the potential to halve the Australian chicken meat industry's soybean meal dependence."

In BCAA 01, elevated dietary leucine inclusions, without or with concomitant increases in isoleucine plus valine, did not enhance growth performance of broiler chickens. Thus, the original hypothesis was rejected. Consequently, trial designs for BCAA 02 and 03 were radically modified, for which permission was given by both AgriFutures Australia and the University of Sydney Ethics Committee.

In BCAA 02, diets with elevated leucine levels or elevated BCAA levels were included in ether wheat- or sorghum-based, CP-reduced diets. In wheat-based diets, elevated BCAA inclusions depressed weight gains by 9.49% and compromised FCR by 8.33% from 7 to 28 days post-hatch. However, elevated leucine and elevated BCAA levels in sorghum-based diets promoted weight gains, both by 9.26%, without altering FCR. The totally opposing performance responses is both extraordinary and unequivocal.

In BCAA 03, L-carnitine (0, 75, 150 mg/kg) was included in sorghum-based diets with CP concentrations of 220, 190 and 160 g/kg. The 75 mg/kg L-carnitine inclusion in 160 g/kg CP diets significantly improved weight gain by 15.0% and FCR by 5.82% from 7 to 33 days post-hatch. In contrast, the effects of L-carnitine in 220 and 190 g/kg CP diets were negligible.

Outcomes/key findings

Essentially, BCAA 01 did not generate any positive outcomes. However, the opposing growth performance outcomes in BCAA 02 is an important finding, as wheat and sorghum are the two dominant feed grains in Australian chicken meat production. This outcome strikingly emphasises the importance of feed grains in reduced-CP diets. Sorghum contains more leucine than wheat; therefore, wheat-based diets contained substantially more non-bound leucine relative to protein-bound leucine. It seems likely that non-bound leucine in wheat-based diets underwent postprandial oxidative losses to greater extents. It has been demonstrated in rats and humans that postprandial oxidative losses in non-bound leucine are greater than in protein-bound leucine (Nolles et al., 2009). Also, BCAA 02 demonstrated that non-bound and protein-bound amino acids are not bioequivalent; a pivotal outcome.

In BCAA 03, growth performance of broiler chickens offered 160 g/kg CP sorghum-based diets was significantly improved by L-carnitine inclusions, which was not the case with 220 and 190 g/kg CP diets. In this study, dietary non-bound amino acid inclusions increased from 15.2 to 29.3 and 51.0 g/kg as CP levels declined. Non-bound amino acids are more likely to undergo hepatic oxidative deamination, which generates ammonia that must be detoxified and excreted as uric acid (Selle et al., 2020b). However, L-carnitine is protective against ammonia intoxication (Kloiber et al., 1988); thus, it appears that L-carnitine facilitated the detoxification of ammonia to enhance growth performance in birds offered 160 g/kg CP diets. The prevention of ammonia intoxication may well be crucial to the development of reduced-CP diets for broiler chickens.

Implications and recommendations

There are tangible implications for the Australian chicken meat industry arising out of this project. The overall objective was to enhance the efficiency whereby dietary protein and amino acids are converted into chicken meat protein. The efficiency of protein utilisation by broiler chickens (33.3%) is superior compared with other terrestrial animals, including pigs (23.3%) and feedlot cattle (12.1%), as assessed by Wu et al. (2014), but would be further improved by reduced-CP diets. It has been estimated that reduced-CP diets would improve the dietary protein-to-carcass protein ratio by 25% from 2.51 to 1.89 (Macelline et al., 2021b). Clearly, the feed grain base of a reduced-CP diet is critically important where starch digestion rates, protein contents and amino acid profiles appear to be key factors. Wheat and sorghum are quite different in these respects.

In BCAA 02, sorghum-based diets generated substantially heavier fat pad weights than wheat-based diets, which may have been a consequence of slower sorghum starch digestion rates. Alternatively, lower protein contents in sorghum than wheat results in lower non-bound amino acid inclusion and a reduced risk of excess amino acid deamination and ammonia intoxication. However, there are nutritional strategies that should be evaluated to prevent the negative impacts of ‘ammonia overload’.

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Publications

The following three publications stemmed directly from this project and have been submitted for publication in a peer-reviewed journal.

Greenhalgh, S., Macelline, S. P., Chrystal, P. V., Liu, S. Y., Selle, P. H. (2021) An evaluation of elevated branched-chain amino acid inclusions on the performance of broiler chickens offered reduced-crude protein, wheat-based diets from 7 to 28 days post-hatch. *Animal Feed Science and Technology* (accepted for publication)

Greenhalgh, S., Macelline, S. P., Chrystal, P. V., Liu, S. Y., Selle, P. H. (2021) Elevated branched-chain amino acid inclusions generate distinctly divergent growth performance response in broiler chickens offered wheat- and/or sorghum-based, reduced-crude protein diets. *Animal Feed Science and Technology* (submitted for publication).

Greenhalgh, S., Hamilton, E. J., Macelline, S. P., Toghyani, M., Chrystal, P. V., Liu, S. Y., Selle, P. H. (2021) Dietary crude protein concentrations and L-carnitine inclusions interactively influence performance parameters of grower broiler chickens offered sorghum-based diets. *Animal Feed Science and Technology* (submitted for publication).

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Dr Peter H. Selle
Poultry Research Foundation
The University of Sydney
425 Werombi Road
Camden NSW 2570

02 9351 1697
peter.selle@sydney.edu.au

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