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EXPLORING GLYCINE AND OTHER AMINO ACIDS IN REDUCED PROTEIN WHEAT DIETS FOR BROILERS

Key information

- Supplementing only essential amino acids failed to maintain performance below 18.3 % crude protein during the grower period (days 7 to 35)
- Glycine plays an important role in broiler nutrition especially in reduced crude protein diets
- The interconversion of glycine and serine is confirmed, with serine supplementation matching that of glycine
- Adding excess threonine in reduced protein diets deteriorates performance
- Protein and amino acids have greater importance during early development up to 21 days of age compared to later phases
- Non-essential amino acids such as aspartic acid and glutamine may become limiting in reduced protein diets
- Increasing amino acid density assists in maintaining performance in birds fed reduced protein diets
- Reducing dietary protein improves nitrogen efficiency, however, breast meat yield was compromised, and relative fat-pad weight increased

Reduced protein diets

Reducing dietary crude protein (CP) has been proactively achieved over many decades with the introduction of amino acid (AA) supplements, however, with further CP reduction the efficiency and productivity of broilers are hindered. This progress has been encouraged by the economics of AA supplements versus protein meals. Additionally, nutritional management of dietary protein has been attributed to improved litter quality and gut health. However, further reduction of CP highlights the delicate balanced practised by nutritionists in obtaining the optimum performance on the least cost diet meeting the minimum nutritional requirements. Nutrients other than essential AA have become the focus of managing reduced protein diets with interest in the non-essential AA, glycine (Gly).

Reduced protein corn-based diets have been heavily researched in poultry, disregarding the portion of the industry that feed wheat-based diets. There is a multifaceted push to reduce the CP of poultry diets to improve sustainability, economics and health and welfare of poultry production. An ever-growing concern about sustainability persistently drives industries to consider the pollution of their production systems, and in poultry, nitrogen has been highlighted as a major contributor to environmental damage (Powers and Angel, 2008). Higher protein diets have been correlated with increased water and nitrogen excretion and deteriorated litter condition (Wheeler and James, 1950; Bregendahl et al., 2002). Poor litter quality impairs bird welfare and health, due to increased occurrence of breast blisters and hock burns (Harms et al., 1977; Shepherd and Fairchild, 2010). Carcass downgrades from these issues can cost up to US $280 million annually (Burgdorfer, 2009). Reducing dietary protein lowers water intake (Alleman and Leclercq, 1997) and decreases the volume of water excreted (James and Wheeler, 1949). Additionally, the impending and current occurrence of antibiotic free diets increases the risk of necrotic enteritis and other intestinal diseases, known to incur US $6 billion in damages annually (Wade and Keyburn, 2015). Drew et al. (2004) correlated increased dietary protein with increased proliferation of *Clostridium perfringens*, a known pathogen of necrotic enteritis (Cooper et al., 2013). A reduction in CP of the diets is expected to improve industry sustainability and bird health and welfare.

Poultry diets typically follow the practice of precision nutrition with formulations meeting specific AA requirements rather than CP. Developing a better understanding of AA profiles in raw materials enables nutritionists to implement reduced protein diets by accurately formulating to specific digestible AA. The increased availability and affordability of crystalline AA has further increased their use resulting in downward pressure on dietary protein content.

At some point of protein reduction, bird growth performance is impaired compared to standard protein diets. Reduced body weight gain (Dean et al., 2006), poorer feed conversion (Fancher and Jensen, 1989a), and reduced breast meat yield (Fancher and Jensen, 1989b; Pesti, 2009) have been reported in comparison to standard protein diets. Reducing dietary CP has also produced inconsistent results in feed intake with both increases (Chrystal et al., 2019) and decreases reported (Namroud et al., 2008). Additionally, increases in whole body fat and relative fat-pad weights have also been reported in reduced protein diets (Fancher and Jensen, 1989a). Reducing dietary CP reduces the inclusion rate of protein meals in favour of AA supplements. Decreasing the inclusion rates of protein meals in poultry diets also reduces the presence of protein meal associated nutrients such as AAs, electrolytes, minerals, and alter starch/fibre dynamics which all may be impacting performance.

Mass production of crystalline AA from bioengineered *Escherichia coli* and C*orynebacterium glutamicum* or by industrial chemical synthesis enables supplementation of AA and reducing CP in poultry diets (D'Este et al., 2018). The first limiting AAs in a typical wheat-based broiler diet are methionine+cysteine, lysine and threonine followed by valine, isoleucine, arginine and Gly, and finally leucine, phenylalanine, histidine and tryptophan. Using crystalline AA to an ideal AA ratio can maintain performance with diets as low as 17 % CP from day 21 to 35 (Belloir et al., 2017). Additionally, supplementing Gly in reduced protein diets has also maintained performance with as low as 16 % CP from day 1 to 17 compared to a standard protein diet, demonstrating the importance of non-essential AA in broiler nutrition (Dean et al., 2006). This highlights the necessity of accurately formulating to specific AA requirements. To further investigate crystalline AA supplementation, including Gly, the authors completed a series of reduced protein wheat-based diet studies at the University of New England, Australia.

Formulating reduced protein diets

Three studies were completed at the University of New England from 2016 to 2018 on Ross 308 males as broilers, off-sex males and breeder males. All studies were approved by the University of New England Animal Ethics Committee (AEC16-050; AEC17-042; AEC18-059). The studies investigated the use of crystalline AA in reduced protein wheat-based diets with a focus on Gly, Gly equivalents and AA ratios. The diets were formulated using AMINOChick® 2.0. software for AA ratios at the appropriate energy levels and chick age. Experimental diets were primarily wheat-based with soybean meal, sorghum and canola oil, diet tables can be found in Hilliar et al., (2019b), Hilliar et al., (In press a), and Hilliar et al., (In press b).

Importance of glycine in reduced protein diets

Performance comparable to that of standard protein diets has been achieved in diets containing 16.0 % CP (Dean et al., 2006; Ospina-Rojas et al., 2014) with the use of crystalline essential AA and Gly. Many studies consider both Gly and serine levels together as Gly equivalence (Gly_{equiv}: Gly + 0.7143 * Ser) in diets due to their interconversion *in vivo* (Baker et al., 1968). The requirement for Gly_{equiv} for optimum growth is yet to be agreed upon. The NRC (1994) provides a requirement of 1.25 % Gly_{equiv}; however, recent literature recommends higher levels varying from 1.50 to 2.44 % (Heger and Pack, 1996; Schutte et al., 1997; Corzo et al., 2004; Dean et al., 2006; Waguespack et al., 2009; Ospina-Rojas et al., 2013a; van Harn et al., 2019) in diets ranging from 16.2 to 22.8 % CP. Heger and Pack (1996) further identified that varying levels of CP can affect the Gly_{equiv} requirement in growing chicks. Additionally, Gly responses are more apparent in younger birds (Ospina-Rojas et al., 2014; Kriseldi et al., 2017), suggesting Gly has greater importance in early development. Apart from structural and functional protein synthesis, Gly is also involved in a variety of metabolic cascades such as creatine and glutathione (Wu, 2013). Furthermore, the link between protein metabolism and Gly is reinforced as one molecule of Gly is required to form each molecule of uric acid synthesised. The net synthesis of Gly cannot meet these requirements alone, making it conditionally essential when insufficient amounts of Gly are offered in the diet, especially when there is an excess of AAs to be deaminated and excreted. Namroud et al. (2008) suggested that appetite impairment in birds fed reduced protein diets was a result of increased blood ammonia (Noda, 1975). The increased blood ammonia was in-turn a result of reduced uric acid synthesis, possibly limited by Gly availability.

The importance of Gly in broiler diets has been evaluated in corn-based diets with little reported in diets based on other grains (Akinde, 2014). This is of interest as the AA profiles of corn- and wheat-based diets differ. Tryptophan for example may be limiting in corn-based diets, however, is one of the last limiting AA in wheat-based broiler diets. Furthermore, the requirement of Gly has been determined to vary depending on dietary CP levels and other AA levels in the diet (Heger and Pack, 1996; Ospina-Rojas et al., 2013b; Siegert et al., 2015a; Siegert et al., 2015b). Therefore, the role of Gly in wheat-based diets must be assessed to test the efficacy of Gly supplementation in reduced protein diets based on this grain. A study was conducted with seven treatments fed to 546 male broilers that included a standard protein (22.5 % CP in grower for d 10 to 21 and 19.7 % CP in finisher for d 21 to 35), reduced protein (20.6/17.8 %), low protein (18.3/16.2 %) and very low protein (17.7/15.5 %) diets without or with Gly supplementation to match that of the standard diets (0.71/0.65 %). Each replicate had 13 individuals per pen with six replicates per treatment. With each level of reduction, performance was severely impacted (Figure 1). Average daily weight gain was reduced by up to 9 % when reducing CP from 22.5 to 17.7 % during the grower phase ($P < 0.001$).

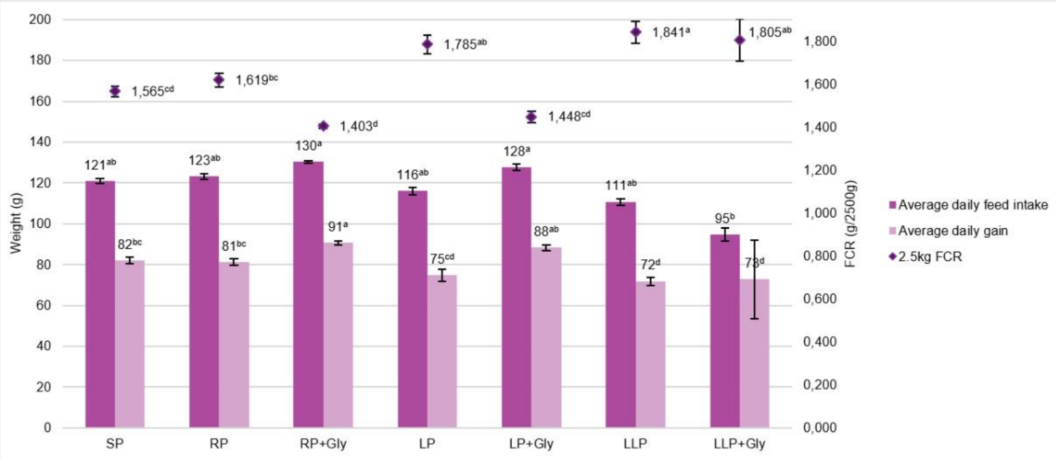

Figure 1: Average daily gain, average daily feed intake and FCR corrected to 2.5kg in study one
SP = standard protein (22.5/19.7 % crude protein), RP = reduced protein (20.6/17.8 % crude protein), LP = low protein (18.3\16.2 % crude protein), LLP = very low protein (17.7/15.5 % crude protein), +Gly = Glycine supplemented to 0.71/0.65 %.

Additionally, daily feed intake also reduced by up to 14 % with feeding a lower protein diet ($P < 0.05$). Correcting the FCR to 2.5 kg body weight showed that reducing protein increased FCR by 28 points ($P < 0.001$). Supplementing Gly improved performance parameters at some CP levels but not all. Glycine supplementation restored or surpassed average daily gains observed in the standard protein treatment at 20.6 and 18.3 % CP; however, no effect was observed in birds fed 17.7 % CP. Similar results were seen in FCR corrected to 2.5 kg body weight as birds fed diets supplemented with Gly had an improved FCR in the first two levels of CP reduction. The Gly_{equiv} recommendation falls between the previous recommendations of between 1.50 to 2.44 %. These results have provided an excellent demonstration of the importance of Gly in reduced protein and confirmed that the AA profile of a wheat-based diet enables similar principles of Gly supplementation derived from corn-based diets.

Glycine alternatives in reduced protein diets

As Gly is a non-essential AA, Gly precursors such as serine and threonine have the potential to meet the Gly_{equiv} requirements in reduced protein diets. After determining the effectiveness of supplementing Gly in reduced protein wheat-based diets, other sources of Gly were evaluated and compared. Serine and Gly share a close metabolic relationship, as the enzyme serine hydroxymethyltransferase facilitates a reversible reaction between them, giving the two AA the abbreviation of Gly_{equiv}. However, threonine, is also degraded to Gly via two separate metabolic cascades; first threonine aldolase degrades threonine directly to Gly, and second a two-step process involving threonine dehydrogenase and glycine C-acetyltransferase which is the major threonine degradation pathway in birds (Kidd and Kerr, 1996). Serine and threonine may thus compensate poor growth which results of Gly deficiency in reduced protein diets. To test the use of Gly precursors in reduced protein wheat-based diets, a study was conducted using 528 off-sex male breeders fed eight dietary treatments. Each replicate had 11 individuals per pen with six replicates per treatment. Two protein levels and two concentrations of Gly_{equiv} with Gly, serine and threonine at 1.6 and 1.8 % Gly_{equiv} were tested. Protein levels were altered between growth phases to reflect industry practices with the standard protein diet at 22.3 and 19.9 % CP and the RP diet at 18.6 and 17.2 % CP for grower (d 7 to 21) and finisher (d 21 to 35) phases respectively. Glycine equivalence using an equimolar conversion to Gly;

1. For serine Gly_{equiv} treatments: Gly (%) + (0.7143 × serine (%))

2. For threonine Gly_{equiv} treatments: Gly (%) + (0.7143 × serine (%)) + (0.6302 × threonine (%) above standard threonine requirement)

Reducing dietary protein from 22.3/19.9 % to 18.6/17.2 % resulted in no differences in performance indicating that the essential AA supplementation in reduced protein diets alone can maintain performance at this CP level (Figure 2). Surprisingly, supplementing 0.84 % threonine to (1.6 % Gly_{equiv}) significantly reduced average daily gain by 7 % and further supplementation to 1.23 % (1.8 % Gly_{equiv}) resulted in a 9 % reduction in average daily gain compared to the non-supplemented reduced protein diet ($P < 0.001$). Comparing Gly and serine supplemented treatments, no significant differences were observed between treatments. Thus, the beneficial effects of Gly supplementation seen in the previous study were not replicated at this CP level. Additionally, the supplementation of serine and threonine to 1.6 % Gly_{equiv} and threonine to 1.8 % Gly_{equiv} impaired FCR (corrected to 2.5 kg body weight) by 17, 21 and 22 points respectively ($P = 0.001$).

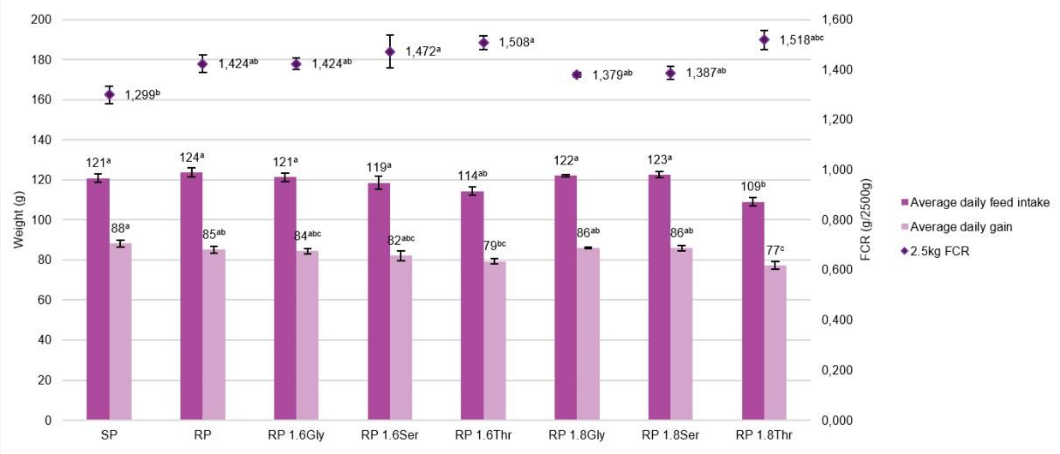

Figure 2: Average daily gain, average daily feed intake and FCR corrected to 2.5kg in study two
SP = standard protein (22.3/19.9 % crude protein), RP = reduced protein (18.6/17.2 % crude protein), 1.6Gly = 1.6 % glycine equivalence with glycine supplementation, 1.6Ser = 1.6 % glycine equivalence with serine supplementation, 1.6Thr = 1.6 glycine equivalence with threonine supplementation, 1.8Gly = 1.8 % glycine equivalence with glycine supplementation, 1.8Ser = 1.8 % glycine equivalence with serine supplementation, 1.8Thr = 1.8 % glycine equivalence with threonine supplementation

The levels of threonine and Gly in the blood plasma (data not shown) increased, respectively, from 1.33 % and 1.99 % in birds fed the non-supplemented reduced protein diet to 3.18 % and 36.29 % respectively in birds fed threonine supplemented to 1.8 % Gly_{equiv} ($P < 0.001$). However, Gly and serine supplemented treatments produced similar plasma AA profiles. The results from this study suggest that threonine and serine are converted to Gly *in vivo*. However, supplementing some threonine can improve performance in reduced protein diets (Ospina-Rojas et al., 2013b), but the consensus is that threonine supplementation cannot satisfy Gly requirements alone (Waldroup et al., 2005; Corzo et al., 2009). This is possibly due to interactions between the diet and threonine dehydrogenase. Birds fed reduced protein diets appear to downregulate genes encoding for hepatic threonine dehydrogenase (Hilliar et al., 2019), but also reduce measured hepatic enzyme activity (Davis and Austic, 1997). Supplementation of threonine on the assumption of a one to one molar basis creates a clear AA imbalance with severe effects on performance. However, the manipulation or supplementation of serine may support Gly_{equiv} deficiencies.

Amino acid densities and reduced protein diets

Reviewing the physical changes in broilers over the past 80 years suggests a greater requirement for AA to achieve maximum efficient growth (Vieira and Angel, 2012). Kidd et al. (2005) found that increasing AA density by approximately 10 % increased body weight gain by 9 % and decreased FCR by 11 points, supporting suggestions by Vieira and Angel (2012) of a greater AA requirement. Furthermore, Liu et al. (2019) found a linear relationship between increasing digestible lysine following an ideal protein ratio and increasing weight gain while reducing FCR.

Increasing the AA density in reduced protein diets, could overcome the poorer performance generally observed while still maintaining some benefits lower CP. A comparison of the roles of non-essentials vs essentials in reduced protein diets must be evaluated as many studies with increased AA densities also increase both essential and non-essentials. Grower and finisher phases were used to reflect industry practices with grower diets fed at d 7 to 21 and finisher diets fed at d 21 to 35. Nine treatments were formulated investigating three protein levels and three AA profiles and fed to 936 Ross 308 male breeders. Each replicate had 13 individuals per pen with eight replicates per treatment. Protein levels were 21.6/19.8 % CP (SP), 19.7/17.5 % CP (RP), and 17.1/15.8 % CP (LP). Amino acid profiles were: 100 % digestible lysine using ideal protein ratio (100 % AA), 115 % digestible lysine using an ideal protein ratio for essential AA with CP equal to 100 % AA (115 % EAA), and 115 % digestible lysine using an ideal protein ratio for essential AA with increased CP (115 % AA). An interaction was observed between protein and AA profile for grower daily gain ($P < 0.001$). Reducing protein reduced daily gain by 7.6 % when comparing SP and LP diets at 100 % AA. However, in LP diets, increasing AA profile from 100 % AA to 115 % AA, daily gain increased by 14.1 %. Daily gain was maintained between all SP and RP diets regardless of AA profile, acknowledging this was achieved with Gly supplementation to 1.6 % Gly_{equiv} (Figure 3). The non-essential AA mostly impacted by the reduction of CP between these treatments was aspartic acid, required for the conversion of glutamic acid from α-ketoglutarate. Glutamic acid plays an important role in nitrogen metabolism, most notably glutamine synthesis, an AA that has demonstrated excellent benefits to performance (Kriseldi et al., 2017; Xue et al., 2018). This highlights the importance of non-essential AA in broiler nutrition and the benefits of increasing AA density with a combination of protein meals and crystalline AA over focusing purely on essential AA.

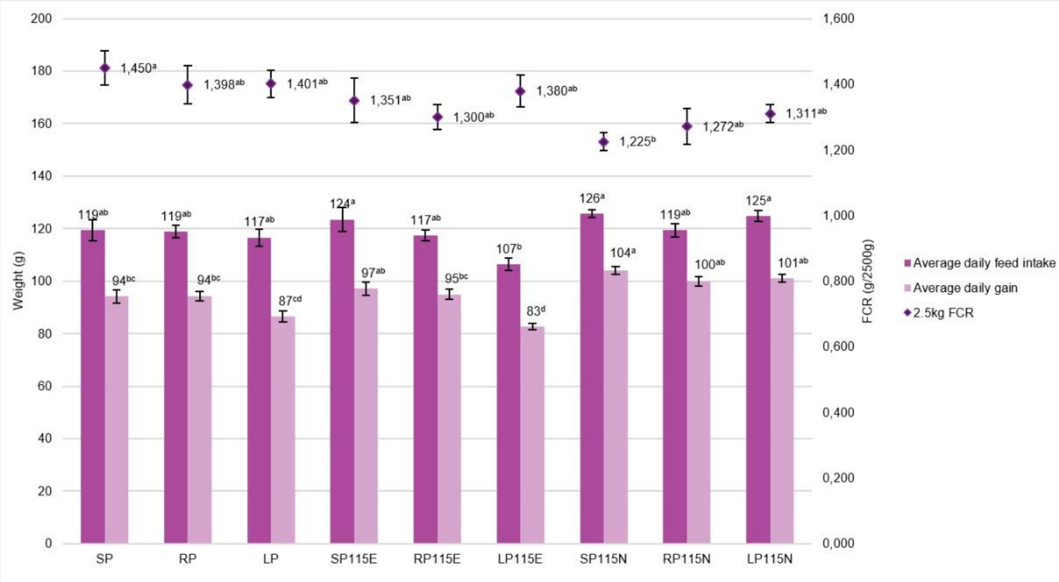

Figure 3: Average daily gain, average daily feed intake and FCR corrected to 2.5kg in study three
SP = standard protein (21.6/19.8 % crude protein), RP = reduced protein (19.7/17.5 % crude protein), LP = low protein (17.1/15.8 % crude protein), 115E = 115 % digestible lysine levels with only essential amino acids increased, 115N = 115 % digestible lysine levels with essential amino acids increased and crude protein.

Early development and amino acids

The results of the first study are not directly comparable to the second and third studies for the grower phase given that the grower phase in the first study was only 11 days compared to 14 days in the second and third studies. Additionally, it must be acknowledged that Ross 308 male broilers were used in the first study, off-sex male breeders in the second and parent breeder males in the third. However, considering the results from the non-supplemented treatments across the three studies highlights the difference between altering the protein in grower and finisher treatments and the role of Gly for growth (Figures 3 and 4). In the first study daily weight gain, daily feed intake and FCR deteriorated without Gly supplementation during the grower period, but no differences in performance were observed between treatments during the finisher period. In the final study no differences in daily weight gain or daily feed intake were observed between treatments in the grower and finisher phases, however FCR deteriorated in the reduced protein treatment compared to the standard protein ($P < 0.05$). Additional inconsistencies exist in the second study as reducing CP resulted in an increase in daily feed intake with detriments to FCR ($P < 0.05$). Protein reduction has a greater negative effect on growth in younger birds (Kriseldi et al., 2017). It is more achievable for the industry to adopt reduced protein diets in grower and finisher phases as the protein is reduced naturally in the diet with age as AA requirement drops (NRC, 1994) and further reductions do not seem to impact performance as greatly as during the younger phases. Additionally, a greater amount of feed is consumed during this period which increases the quantifiable benefits of reduced protein diets.

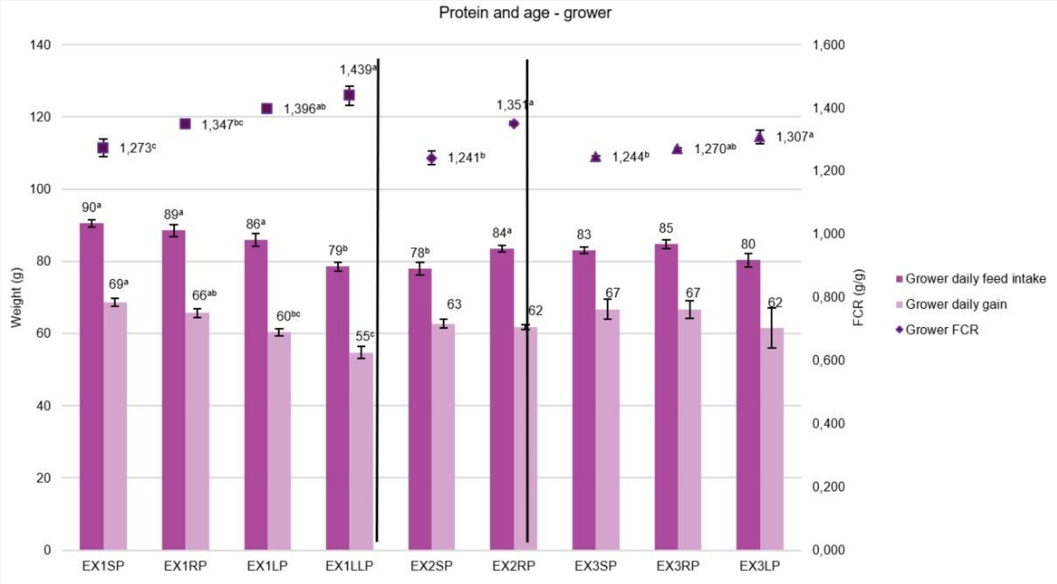

Figure 4: Average daily gain, average daily feed intake and FCR for the grower period for non-supplemented treatments across all studies EX1SP = Experiment 1 standard protein, EX1RP = Experiment 1 reduced protein, EX1LP = Experiment 1 low protein, EX1LLP = Experiment 1 very low protein, EX2SP = Experiment 2 standard protein, EX2RP = Experiment 2 reduced protein, EX3SP = Experiment 3 standard protein, EX3RP = Experiment 3 reduced protein, EX3 LP = Experiment 3 low protein.

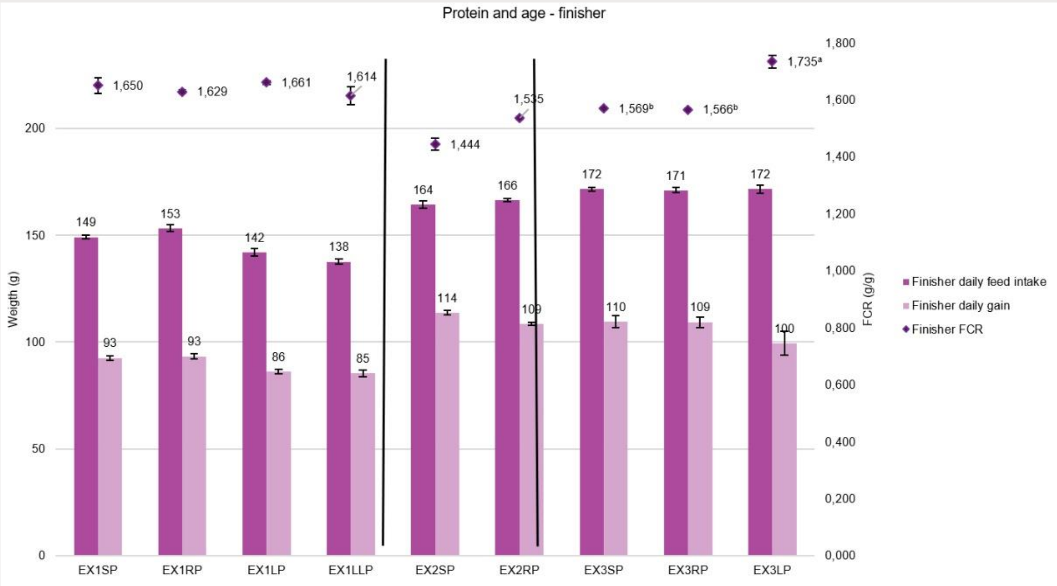

Figure 5: Average daily gain, average daily feed intake and FCR for the finisher period for non-supplemented treatments across all studies EX1SP = Experiment 1 standard protein, EX1RP = Experiment 1 reduced protein, EX1LP = Experiment 1 low protein, EX1LLP = Experiment 1 very low protein, EX2SP = Experiment 2 standard protein, EX2RP = Experiment 2 reduced protein, EX3SP = Experiment 3 standard protein, EX3RP = Experiment 3 reduced protein, EX3 LP = Experiment 3 low protein.

Nitrogen efficiency and body composition

A push for reduced protein diets comes from environmental concerns, especially the amount of nitrogen effluent entering the environment from agricultural production systems. Legislation has been brought into the European Union to monitor pollution from nitrogen effluent, bringing in legislative and financial pressures to reduce it. The lifecycle of nitrogen in poultry production systems starts in the form of protein in the diet and reducing dietary protein provides an option to reduce nitrogen effluent. Nitrogen efficiency is a calculation that provides an estimate on how efficiently nitrogen is utilised within the body. Nitrogen consumed per kg of body weight gained is calculated assuming every kilogram of live weight in poultry contains 29 g of nitrogen. An increased nitrogen efficiency indicates improved nutrient utilisation per unit of body weight produced. Considering the data pertaining to CP consumed against the constant for nitrogen in body weight gain, the nitrogen efficiency of birds can be determined following calculations of Belloir et al. (2017). In all three treatments a positive correlation was observed between reducing dietary protein and increasing nitrogen efficiency. Study three investigated the greatest range in dietary CP and showed a strong correlation between reducing CP and increase nitrogen efficiency during the grower phase (R^2 = 0.90), however, this correlation deteriorated in the finisher phase (R^2 = 0.57).

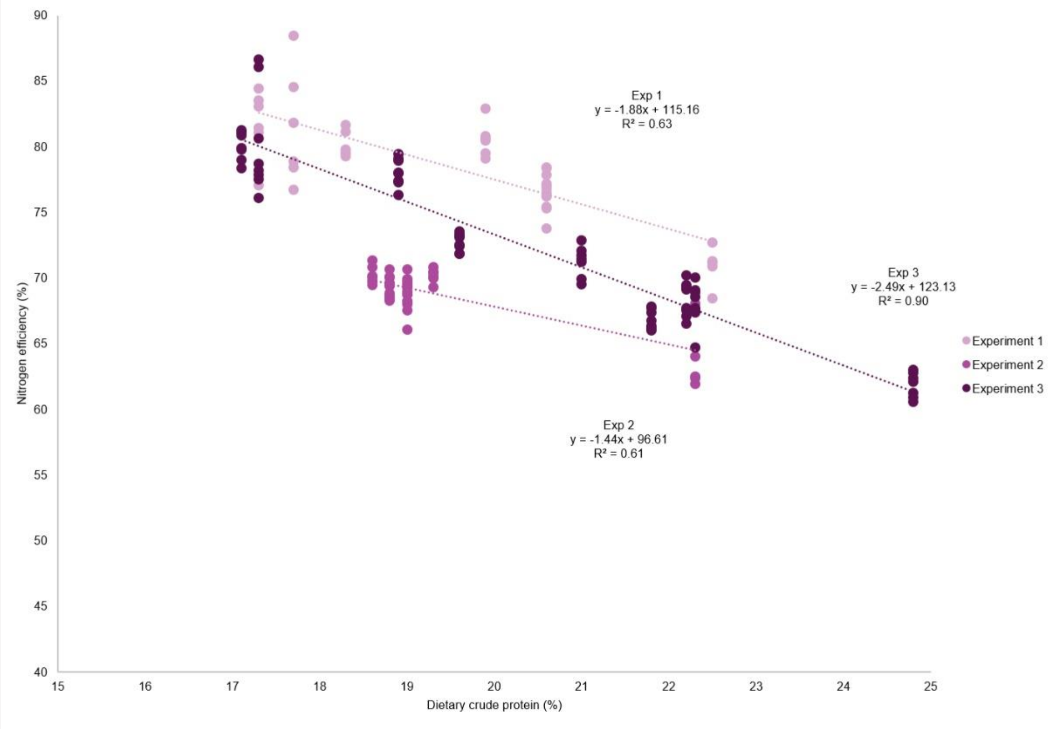

Figure 6: Nitrogen efficiency across all treatments and studies during the grower period

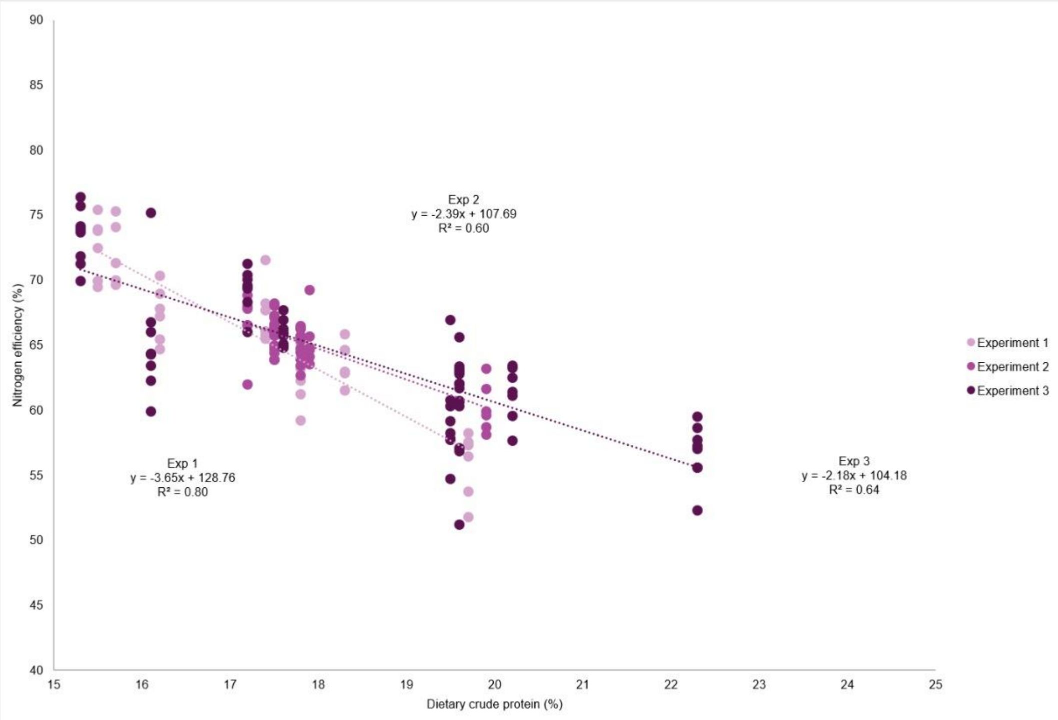

Figure 7: Nitrogen efficiency across all treatments and studies during the finisher period

While there were positive correlations across the studies between CP and nitrogen efficiency, the constant of 29 g/kg live weight for whole body nitrogen must be reviewed. Reducing dietary protein increases relative fat-pad weight, and it is proposed that the percentage of body nitrogen retained is not constant in birds fed reduced protein diets. Across these studies an increase in relative fat-pad weight was observed when reducing dietary protein (Figure 8), and in the final experiment relative liver weights increased (Hilliar et al., in press). These results suggest that while the birds appear to be more nitrogen efficient when reducing CP, essentially, the data are not accurate and more in-depth analysis is required.

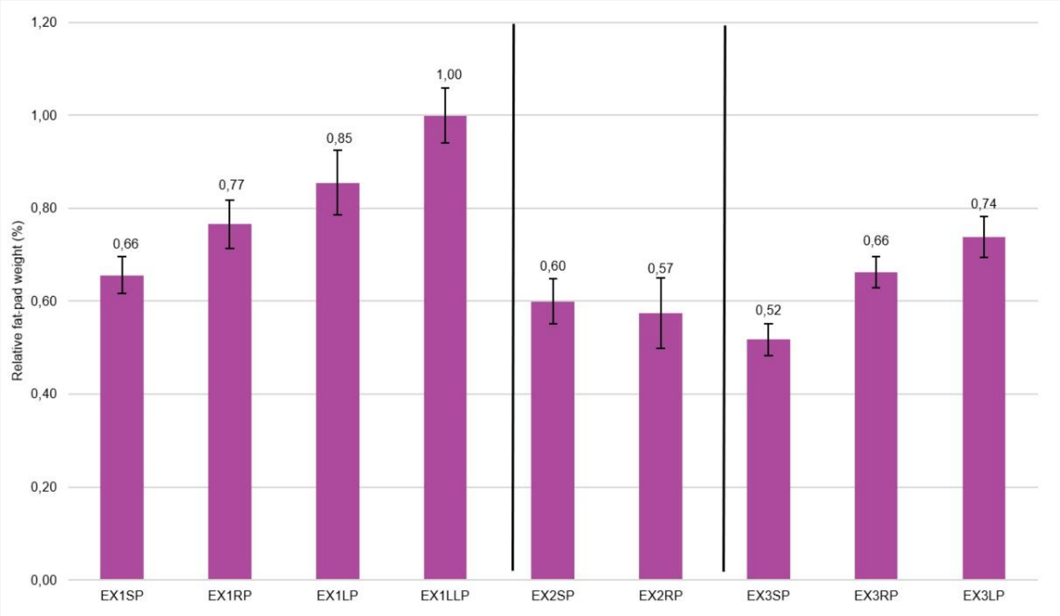

Figure 8: Relative fat-pad weights for non-supplemented treatments across all treatments
EX1SP = Experiment 1 standard protein, EX1RP = Experiment 1 reduced protein, EX1LP = Experiment 1 low protein, EX1LLP = Experiment 1 very low protein, EX2SP = Experiment 2 standard protein, EX2RP = Experiment 2 reduced protein, EX3SP = Experiment 3 standard protein, EX3RP = Experiment 3 reduced protein, EX3 LP = Experiment 3 low protein.

Conclusions

Reducing CP in meat chicken diets contributes to improved environmental sustainability and improved health and welfare. However, reducing dietary protein in current Australian diets without nutrient balancing has a significant effect on performance, particularly in younger birds. This deterioration in performance can be overcome with supplementation of essential AA, including Gly, however an increase in body fat and fat-pad may still occur. In the wheat-based diets investigated, using threonine to cover Gly deficiencies may further impair performance in birds fed reduced protein diets, however, mitigating serine offers some relief to Gly deficient diets. Increasing the AA density of the diet is another aspect to improve performance, however, sacrificing non-essentials in favour of essentials also hinders performance. Reduced protein diets are currently feasible in finisher diets where AA requirements are lower, AA have less of an effect on performance and the identified benefits of reduced protein diets are greatest. The implementation of reduced protein diets will require a multi-faceted approach including consideration of all twenty AA, other protein-related nutrients and the digestibility and interactions of raw materials.

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Acronyms

CP crude protein, AA amino acids, Gly glycine, Gly_{equiv} glycine equivalents.

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