Facilitating the acceptance of tangibly reduced-crude protein diets for chicken-meat production

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Abstract

Inclusions of non-bound amino acids particularly methionine, lysine and threonine, together with the “ideal protein” concept have allowed nutritionists to formulate broiler diets with reduced crude protein (CP) and increased nutrient density of notionally “essential” amino acids and energy content in recent decades. However, chicken-meat production has been projected to double between now and 2050, providing incentives to reduce dietary soybean meal inclusions further by tangibly reducing dietary CP and utilising a larger array of non-bound amino acids. Whilst relatively conservative decreases in dietary CP, in the order of 20 to 30 g/kg, do not negatively impact broiler performance, further decreases in CP typically compromise broiler performance with associated increases in carcass lipid deposition. Increases in carcass lipid deposition suggest changes occur in dietary energy balance, the mechanisms of which are still not fully understood but discourage the acceptance of diets with reductions in CP. Nevertheless, the groundwork has been laid to investigate both amino acid and non-amino acid limitations and propose facilitative strategies for adoption of tangible dietary CP reductions; consequently, these aspects are considered in detail in this review. Unsurprisingly, investigations into reduced dietary CP are epitomised by variability broiler performance due to the wide range of dietary specifications used and the many variables that should, or could, be considered in formulation of experimental diets. Thus, a holistic approach encompassing many factors influencing limitations to the adoption of tangibly reduced CP diets must be considered if they are to be successful in maintaining broiler performance without increasing carcass lipid deposition.

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1. Introduction

A tangibly reduced-crude protein (CP) diet for broiler chickens reared from 14 to 35 d post-hatch contains between 2% and 3% less CP compared to a conventional diet. The formulation of these diets is typically based on decreases in soybean meal and increases in feed grain (maize or wheat) contents coupled with elevated inclusions of non-bound (crystalline and synthetic) amino acids to meet requirements. Real benefits for sustainable chicken-meat production using less resources will stem from the successful development of such diets. These advantages range from reduced nitrogen and ammonia emissions, improved litter quality and enhanced bird welfare to less undigested protein passing into the hind gut to fuel the proliferation of potential pathogens (Greenhalgh et al., 2020). Whilst greater reductions of dietary CP (40 to 50 g/kg) invariably compromise broiler performance and increase lipid deposition, a limited number of studies have investigated both aspects. Comparing reported data is problematic due to different bird strains, genders, ages and dietary nutrient concentrations used. Furthermore, body lipid is often recorded as total body lipid or weight of abdominal fat pad rather than relative fat pad weights. Where data on body weight rather than weight gain...
was available, the latter measure may be calculated and the data from 19 studies is presented (Table 1). Collectively, data suggest that a reduction in dietary CP of 55 g/kg (172 vs. 227 g/kg) depressed weight gain by 5.1%, increased feed conversion ratio (FCR) by 9.0% and increased fat deposition by 36%. Thus, inferior FCR and increased fat deposition epitomise the challenges to successfully reducing dietary CP using substantial levels of non-bound amino acids.

Nevertheless, it has been demonstrated that moderate dietary CP reductions (20 to 30 g/kg) without inferior broiler performance and profitability are achievable. This was demonstrated in Van Harn et al. (2017) where reductions in CP of 22 and 23 g/kg in the growing and finishing phase of Ross 308 male broilers did not influence live weight gain and feed intake but significantly improved FCR by 3.5% (from 1.555 to 1.500; P < 0.05). However, there is a lack of information on effect size and power analysis in studies reported in the literature; therefore, small but economically important impact on growth performance may not be detected in these studies. More recently, dietary CP reductions in the order of 30 g/kg whilst maintaining broiler performance have been possible in modern broiler diets and substantially decrease dietary soybean meal inclusion by over 30%. Furthermore, N excretion declines by approximately 14% providing strong environmental incentives to successfully reducing CP in broiler feeds by greater than 30 g/kg (Kriseldi et al., 2018; Chrystal et al., 2020a; Van Harn et al., 2019).

Almquist and Grau (1944) appear to be the first investigators to observe that birds offered broiler diets supplemented with non-bound amino acids were incapable of maintaining growth performance. Such findings imply that there is a threshold to CP reductions that can be accommodated by broiler chickens. If the factors contributing to this threshold were to be identified it should be possible to put corrective strategies in place so that tangibly reduced-CP diets, with their attendant advantages, will meet acceptance. Several reasons have been advanced for the poor performance of broilers offered tangibly reduced-CP diets. These include the inherently different digestive dynamics of ‘intact’ protein, di- and tri-peptides and non-bound amino acids and direct amino acid limitations. These limitations may include an insufficient nitrogen (N) pool for synthesis of non-essential amino acids, dietary imbalances between notionally essential and non-essential amino acids and the distinct possibility that amino acid requirements have not been identified with sufficient accuracy in the context of reduced-CP diets (Waldroup, 2007; Siegert et al., 2015b; Chrystal et al., 2020b). However, some groundwork has been laid for a better understanding of feeding reduced CP diets to broilers with respect to amino acid requirements, antagonisms, and imbalances (Kidd et al., 2013; Wu, 2014; Kidd and Choct, 2017).

The use of non-bound or feed-grade amino acids in linear least-cost feed formulation started in 1950s with the rapid adoption of methionine in poultry diets; however, the second limiting (lysine) and third limiting (threonine) amino acid did not occur until the 1970s and 1990s (Kidd et al., 2013). The gradual acceptance of non-bound methionine, lysine and threonine in diet formulations, together with the “ideal protein” concept has allowed nutritionists to formulate broiler diets at far lower dietary CP contents than diets containing only ‘intact’ protein meals to meet amino acid requirements. Formulating maize-soybean meal-based broiler starter diets without non-bound methionine, lysine and threonine, has been shown to increase CP from 200 to 356 g/kg and soybean meal inclusion from 293 to 709 g/kg (Pesti, 2009). Therefore, the purpose of this review is to consider the key amino acid and non-amino acids factors limiting the adoption of tangibly reduced CP diets and to propose strategies that may overcome these limitations.

2. Background

The global demand for chicken-meat has been projected to increase from 105.6 million tonnes at present to 181.3 million tonnes in 2050 which is an increase of 72% (Alexandratos and Buurma, 2012). Inevitably, this will increase the demand for poultry feed including soybean meal and cereal grains. Indeed, Masuda and Goldsmith (2008) factored in soybean yield improvements from approximately 2.5 to 3.0 tonnes per hectare and concluded that an extra 1.09 million hectares of arable land will be required annually to sustain demand for soybeans from 2008 to 2030. However, the

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1. FCR is expressed in grams of feed per gram of live weight gain.
2. Body lipid as a percentage of body weight (%).
3. Body lipid as relative abdominal fat pad weight in grams per kilogram of body weight (g/kg).

Table 1

| Reference              | Sex     | Age, d | CP, g/kg | Live weight gain, g | FCR, g/kg | Body lipid |
|------------------------|---------|--------|----------|---------------------|-----------|-----------|
|                        |         |        | LCP diet | HCP diet            | LCP       | HCP       | LCP HCP   |
| Jackson et al. (1982)  | Mixed   | 0 to 49| 160      | 360                 | −         | −         | −         | 31.2       | 14.9       |
| Bedford and Summers (1985) | Male  | 0 to 21| 140      | 220                 | 322       | 400       | 2.033     | 1.667      | 16.2       | 12.1       |
| Edmonds et al. (1985)  | Male    | 8 to 16| 160      | 240                 | 109       | 139       | 1.923     | 1.462      | 15.6       | 11.3       |
| Summers and Leeson (1985) | Male  | 0 to 28| 200      | 240                 | 873       | 947       | 1.840     | 1.620      | 21.0       | 17.0       |
| Fancher and Jensen (1989a) | Male  | 21 to 42| 160     | 215                 | 1146      | 1302      | 2.084     | 1.859      | 21.5       | 15.4       |
| Fancher and Jensen (1989b) | Female | 21 to 42| 164     | 194                 | 922       | 923       | 2.288     | 2.183      | 23.4       | 20.4       |
| Moran et al. (1992)    | Male    | 0 to 42| 193      | 224                 | 2042      | 2077      | 1.940     | 1.880      | 17.1       | 14.4       |
| Kidd and Kerr (1996)   | Mixed   | 21 to 42| 168     | 200                 | 1323      | 1386      | 2.110     | 2.040      | 23.8       | 19.9       |
| Aletor et al. (2000)   | Male    | 22 to 42| 153     | 225                 | 1539      | 1522      | 1.920     | 1.800      | 10.6       | 7.1        |
| Erwan et al. 2009      | Male    | 21 to 42| 180     | 200                 | 1170      | 1226      | 1.920     | 2.130      | 12.4       | 11.3       |
| Corzo et al. (2011)    | Male    | 21 to 42| 189     | 202                 | 2030      | 2050      | 1.830     | 1.770      | 15.7       | 13.5       |
| Belloroi et al. (2017, Exp. 1) | Male   | 21 to 35| 150     | 190                 | 1478      | 1479      | 1.710     | 1.640      | 21.5       | 21.6       |
| Belloroi et al. (2017, Exp. 2) | Male   | 21 to 35| 160     | 190                 | 1374      | 1338      | 1.710     | 1.680      | 21.5       | 19.3       |
| Kriseldi et al. (2017) | Male    | 0 to 41| 196      | 220                 | 3057      | 3072      | 1.605     | 1.575      | 11.9       | 8.5        |
| Liu et al. (2017)      | Male    | 7 to 28| 154      | 400                 | 828       | 1450      | 2.206     | 1.139      | 36.9       | 10.2       |
| Sigolo et al. (2017)   | Male    | 0 to 42| 202      | 207                 | 1916      | 2250      | 1.754     | 1.709      | 19.0       | 14.3       |
| Rehman et al. (2018)   | Male    | 0 to 42| 188      | 193                 | 2143      | 2329      | 2.230     | 2.140      | 23.9       | 24.8       |
| Shao et al. (2018)     | Male    | 22 to 56| 170     | 190                 | 2027      | 2020      | 2.540     | 2.580      | 15.1       | 11.6       |
| Srialatha et al. (2018)| Male    | 0 to 42| 182      | 202                 | 2075      | 1899      | 1.750     | 1.600      | 22.0       | 15.9       |
extra land required over the past 10 years has been closer to 5.6 million hectares annually and the projection for 2030 soybean production has already been exceeded (http://statistics.amis-outlook.org/data/index.html). Thus, the successful development of tangibly reduced CP broiler diets is important for sustainable chicken meat production.

Additional benefits of reduced dietary CP include a reduction in water intake and improved litter conditions leading to an improvement in bird welfare issues with reduced incidences of foot pad dermatitis and breast blisters (Garland, 2018; Lemme et al., 2019). Furthermore, a reduction in dietary CP may improve flock health by reducing the risk of necrotic enteritis (NE) caused by the proliferation of Clostridium perfringens in the hind gut (Drew et al., 2004; Wilkie et al., 2005). The reduced usage of in-feed antibiotics has resulted in a resurgence of interest in understanding the pathogenesis of necrotic enteritis and investigating how it can be prevented by alternative interventions including the manipulation of dietary CP (Mot et al., 2014; Rodgers et al., 2015; Prescott et al., 2016).

The economic benefits of reducing dietary CP stem from reductions in energy expenditure on excreting excess N as uric acid and sparing of matrix space in feed formulation for inclusion of less energy dense ingredients, potentially reducing feed costs (Kidd and Choct, 2017). As non-bound amino acids become more economically feasible a greater array of these amino acids will be included in commercial diets at increasing inclusion levels and dietary CP and soybean meal contents will continue to decline.

3. Protein

“Crude protein” has been used for over 150 years and is simply the N content of the feed multiplied by 6.25, based on the assumption that most proteins contain 165 N (Jones, 1941). Thus, CP is somewhat misleading and it does not give any indication of the quality of the protein (Lemme, 2018). However, the majority of practical commercial broiler diets are formulated to digestible (mainly essential) amino acids and CP as a measure of dietary N is justified. Much of the research into reduced-CP diets do not define the CP values that have been attributed to non-bound supplemental amino acids (Aftab et al., 2006). Presumably, the reasons for not assigning CP values to non-bound amino acids are due to the actual values this assigns to the individual amino acid. For example, in some cases amino acid CP exceeds 1,000 g/kg when the factor of 6.25 is applied including glycine (1,154 g/kg CP) and L-arginine (1,996 g/kg CP). However, with the exception of L-lysine HCl (and L-lysine sulphate), the balance of the commercially available non-bound amino acids has a CP value below their true protein (amino acid) value.

Between 1960 and 2000, the “ideal protein” concept was developed by Mitchell, Scott and Baker for meat-type chickens (and pigs) at the University of Illinois, USA. The patterns of dietary essential amino acids were based on tissue proteins and included all the essential amino acids that are not synthesised de novo in animals, but excluded requirements for non-essential amino acids due to limited knowledge of their metabolism and function (Wu, 2014). The requirements of individual amino acids and the development of the ideal protein concept, where the nominally “essential” amino acids are expressed in a ratio to the reference amino acid, lysine, were published by Baker and Han (1994). Similar ideal protein ratios have been suggested by numerous other researchers including Lemme et al. (2006), Tillman and Dozier (2013), Wu (Chort, 2014) and Rostagno et al. (2017). Wu (2014) appears to be the only researcher who included non-essential amino acids in the Texas A&M “ideal protein” concept, although Alhotan and Pesti (2016) by implication set a minimum non-specific non-essential amino acid ratio by stipulating a minimum digestible lysine to true protein ratio. However, it is probable that ideal amino acid ratios will be quite different in tangibly reduced-CP diets.

Early work by Grau (1948) suggested that the lysine requirement for maximum growth at a particular CP level increases as dietary CP increases. However, in a review of a series of papers published between 1987 and 1992 from Reading and Natal Universities it was concluded that excess CP, in rather modest amounts, can depress the utilisation of the first limiting amino acid in growing chicks (Morris et al., 1999). Additionally, the maximum growth rate response, based on relative gain per day (%) declined as dietary CP reduced. The optimum essential amino acid-N to total amino acid-N ratio for maximum weight gain for rats, pigs and poultry suggested by Heger (2003) were similar, varying between 0.55 and 0.60. Substantive reductions in dietary CP seek to challenge this paradigm by increasing digestible amino acid ratios to reduced dietary CP.

4. Amino acid digestibility

In the quest to develop tangibly reduced-CP diets scant attention has been paid to apparent amino acid digestibility coefficients. Indeed, the only previous study we are aware of is that of Awad et al. (2016) who reported the effects of low protein diets on ileal amino acid digestibilities in broiler chickens raised under tropical conditions. These researchers found a significant 6.18% increase (0.790 vs. 0.744) in average ileal amino acid digestibility coefficients pursuant to a reduction in dietary CP from 210 to 165 g/kg. However, in 3 consecutive assays (Chrystal et al., 2020a, 2020b, 2020c) increases in amino acid digestibilities were consistently observed following reductions in dietary CP of maize-based diets but the magnitude of these increases was variable. The most pronounced response was observed in Chrystal et al. (2020a), where the transition from 210 to 165 g/kg CP triggered linear increases in the jejunal digestibilities of 17 amino acids (r = -0.556; P < 0.005) by 29%. In wheat-based diets, Hilliar et al. (2020) reported an average increase of 9.10% in the ileal digestibility of 16 amino acids following the transition from 200 to 170 g/kg CP. These substantial variations in amino acid digestibility coefficients indicate they may trigger post-enteral amino acid imbalances in birds offered reduced-CP diets. The likelihood is that the genesis of these variable, but at times pronounced, increases in apparent amino acid digestibility coefficients stems mainly from attenuated flows of endogenous amino acids. It follows that reduced-CP diets containing less ‘intact’ protein would diminish the secretion of pepsin, trypsin and the remaining endogenous proteolytic enzymes and, in turn, mucin which is a prolific source of endogenous amino acids (Lien et al., 2001). Reduced endogenous amino acid flows would increase apparent digestibility coefficients. The increase in apparent digestibility is also consistent with the premise that free, non-bound amino acids are rapidly absorbed (Wu, 2009) and are notionally 100% digestible (Lemme et al., 2005).

Several recognized amino acid limitations hinder the adoption of reduced-CP diets. However, our contention is that additional consideration should be directed towards threonine and the conditionally essential amino acids, glycine and serine, the branched-chain amino acids (BCAA) and the aromatic amino acids. Consequently, these 3 topics are addressed in the next section.

5. Amino acid considerations

5.1. Glycine, serine and threonine

Glycine is notionally a non-essential amino acid although glycine may become conditionally limiting in reduced CP diets.
(Siegert et al., 2015a, 2016, 2015b; Hilliar et al., 2017b). In Kriseldi et al. (2017) the effects of glycine equivalents (Glyequi) and glutamine supplementation on growth performance and carcass characteristics of male Ross 708 broilers reared on reduced CP diets to 42 d post-hatch were investigated. These authors concluded that providing sufficient dietary glycine plus serine may be necessary to maintain broiler performance when dietary CP was reduced by approximately 24 g/kg.

Dean et al. (2006) described Glyequi as the sum of glycine and the molar equivalent of serine and the interconversion of these is considered to be unrestricted in poultry.

Glyequi may be calculated from the following equation:

\[ \text{Glyequi (g/kg)} = \text{Glycine (g/kg)} + [\text{Serine (g/kg)} \times 0.7143] \]

Serine is required for the conversion of methionine into cysteine and reducing dietary CP increases supplementation of d,L-methionine and usually ignores specific requirements for cysteine which may be important. Each molecule of methionine not converted to cysteine reduces the requirement for Glyequi. As a result, during the conversion, ammonia is produced and one molecule of glycine is required for the de novo synthesis of one molecule of urea in the excretion of ammonia-N. It has been suggested that responses to Glyequi will vary depending upon the CP content of the diet (Siegert et al., 2015b; Siegert and Rodehutscord, 2015; Hilliar et al., 2017). In a meta-analysis of 10 studies, Siegert et al. (2015b) concluded that Glyequi had significant positive effects on weight gain, feed intake and feed conversion efficiency (FCE). Also, that methionine to methionine plus cysteine ratios were important in maximising responses in FCE with Glyequi. Data from the Siegert et al. (2015b) meta-analysis is provided in Tables 2 and 3.

Somewhat surprisingly, threonine was not included as a variable in Siegert et al. (2015b) as the authors reasoned that dietary variations in threonine were low and that most studies had essential amino acids meeting or exceeding NRC (1994) requirements. Increasing glycine plus serine from 15.5 to 16.5 g/kg in a 182 g/kg CP diet increased weight gain by 4.94% from 21 to 42 d post-hatch in broilers offered diets containing 5.7 g/kg digestible threonine. However, when diets contained 6.5 g/kg digestible threonine, the addition of glycine plus serine depressed weight gain by 3.21% in Corzo et al. (2009). Thus, additional threonine compromised responses to Glyequi. In contrast, Chrystal et al. (2020c) observed that in tangibly reduced CP (165 g/kg) maize-soybean meal diets, broiler growth performance was improved from the combined additions of threonine and glycine plus serine which was not the case when threonine or glycine plus serine were added individually to the reduced-CP diet. This is in agreement with Ospina-Rojas et al. (2013) who observed that increasing threonine from 7.0 to 7.7 g/kg in a broiler diet containing 14.7 g/kg glycine plus serine increased weight gain by 3.21%, decreased feed intake by 3.26% and improved FCR by 6.59% from 21 to 35 d post-hatch. Clearly, interactions between threonine with glycine plus serine should not be overlooked and may explain some of the variation in results reported in the literature.

Additionally, glycine can be metabolised from choline when l-homocysteine is available, through a 5-step process, or from threonine directly via threonine dehydrogenase or threonine aldehyde (Siegert et al., 2015a). However, in an instructive study, Yin et al. (2020) offered reduced dietary CP from 215 to 165 g/kg to male, off-sex Ross 308 broilers from 7 to 35 d post-hatch. Free amino acid concentrations were determined in the portal and systemic blood plasma and, whilst portal levels were higher, patterns were similar. The reduction in dietary CP generated an average 30.9% increase in glutamine levels that may be a result of increased condensation of ammonia plus glutamic acid into glutamine. Glyequi decreased by 23.6% which may reflect elevated demand for glycine and serine into the Krebs uric acid cycle. In contrast, average threonine concentration rose by 28.0% which is not indicative of threonine being a precursor of glycine in broiler chickens.

Dietary choline should also be considered and a study to provide 80 mg/d intake in broilers observed FCE and average daily gain (ADG) varied based on intakes of Glyequi and threonine (Siegert et al., 2015a). Increasing threonine intake reduced the Glyequi required to achieve targeted weight gains and linear FCR. Also, dietary choline should always be present in sufficient quantities in studies involving the threonine–glycine–serine axis so that it is not a confounding factor.

Glycine is an amino acid readily susceptible to early Maillard reactions with glucose by Hodge (1953) forming intermediate Amadori compounds to reduce amino acid availability and subsequent digestibility. Typical broiler diets contain in the order of 35 to 45 g/kg sugars. This is relevant given most broiler diets are steam-conditioned and pelleted between 80 and 90 °C which may partly explain why broiler growth responses to Glyequi reduced CP diets are variable if glycine becomes involved in Maillard reactions. However, there is a paucity of data on the effect of steam conditioning at high temperatures on reduced CP diets containing high levels of non-bound amino acids and further research into these possible chemical reactions is warranted, particularly if the diets contain added dextrose and non-bound glycine.

### 5.2. Branched-chain amino acids: leucine, isoleucine and valine

In addition to serving as a “building block” for protein synthesis, leucine has been shown to regulate both protein and lipid metabolism, promoting lean tissue gain in young animals and alleviating muscle protein loss in aging adults and food-deprived animals (Hundal and Taylor, 2009; Columbus et al., 2015; Duan et al., 2015). Indeed leucine was identified as a possible regulator of protein turnover in muscle decades ago (Buse and Reid, 1975). Moreover, it is relevant that high dietary leucine levels have been shown to

### Table 2

Parameter estimates and goodness of fit criteria for the mixed model fitted to broiler response traits using Glyequi as an independent variable over 6 experiments.\(^1\)

| Model                  | ADG, g/d       | ADFI, g/d      | FCE, g/g      |
|------------------------|----------------|----------------|--------------|
|                         | Coefficient   | SE             | P-value      | Coefficient | SE             | P-value      | Coefficient | SE             | P-value      |
| Intercept              | 18.723        | 3.855          | 0.0046       | 38.704      | 5.317          | 0.0008       | 0.488       | 0.037          | <0.0001      |
| Glyequi                | -3.371        | 0.857          | 0.0002       | -2.027      | 1.023          | 0.0518       | -0.047      | 0.012          | 0.0002       |
| Glyequi\(^2\)          | -0.122(SE0.866) + 0.004(SE0.026)x | y = -0.100(SE0.913) + 0.002(SE0.021)x | y = -0.011(SE0.040) + 0.014(SE0.053)x |
| R\(^2\)                | 0.956         | 0.972          | 0.843        | 0.956       | 1.550          | 0.018        |
| Root MS error          | 1.300         | 1.550          |             | 1.300       | 1.550          |             |

Glyequi = glycine equivalents; ADG = average daily gain; ADFI = average daily feed intake; FCE = feed conversion efficiency; SE = standard error; MS = mean square.

*Residual against prediction; none of the slopes were significantly different from 0 (P > 0.05).

\(^1\) Adapted from Siegert et al. (2015b).
activate the mammalian target of rapamycin (mTOR) signalling pathways that stimulate protein synthesis in young broiler chickens (Deng et al., 2014).

Practical wheat-based broiler diets exceed the minimum ideal protein ratio of leucine to lysine and this is even more pronounced with maize and sorghum-based broiler diets. Diets that include raw materials such as maize gluten, maize dried distillers’ grains plus solubles and blood meal that are high in leucine will only increase leucine excess (Waldroup et al., 2002; Rostagno et al., 2011). Optimal ratios of lysine to leucine, valine and isoleucine of 109, 80 and 69, respectively, were proposed by Wu (2014). However, in modern practical broiler diets, with small decreases in dietary CP of 10 g/kg, leucine to lysine ratios of between 120 and 130 in wheat-soybean meal-based diets and 150 to 170 in maize-soybean meal-based diets are quite standard. Thus, by default the minimum “ideal” protein ratio is achievable for valine and isoleucine but leucine is usually supplied in excess.

Leucine-induced BCAA antagonism was first observed in animals decades ago as the addition of L-leucine (30 g/kg) to a low-protein (90 g/kg casein) diet was found to cause marked growth depression in rats that could be partially overcome by supplementation with isoleucine (Harper et al., 1984). Antagonistic BCAA interactions have been reported to change BCAA concentrations in blood plasma and body tissues whilst high intakes of leucine depress valine and isoleucine concentrations in blood and muscle (Harper et al., 1984). The antagonistic effect of disproportionate amounts of BCAA on broiler and rat performance has been well documented; excess leucine disrupts the utilisation of isoleucine and valine, especially when these 2 amino acids are marginal or limiting (Harper, 1956; Harper et al., 1970; D’Mello and Lewis, 1970; Boldizar et al., 1973; Smith and Austic, 1978; Burnham et al., 1992). For broiler chickens offered a valine deficient diet (6.3 g/kg) with adequate levels of isoleucine and valine, poor weight gains were associated with a high incidence of feather and leg abnormalities. However, birds offered diets adequate or deficient in total BCAA did not exhibit these signs and it was proposed by Farran (1987) that the BCAA requirement for broilers is influenced by BCAA antagonism.

Dietary levels of 11.6 g/kg for leucine, 9.0 g/kg for valine and 7.8 g/kg for isoleucine have been proposed to support maximum weight gain and FCE in male broilers to 21 d post-hatch (Farran and Thomas, 1990). More recently, Ospina-Rojas et al. (2017) comprehensively investigated responses of BCAA in Cobb 500 male broilers from 21 to 42 d post-hatch. A basal maize-soybean diet with reduced CP was formulated according to the nutritional recommendations for male broiler chickens proposed by Rostagno et al. (2011) excluding digestible leucine and valine levels. A factorial treatment array consisted of 5 digestible leucine levels (10.0, 12.0, 14.0, 16.0, or 18.0 g/kg) and 5 digestible valine levels (5.2, 6.7, 8.2, 9.7, or 11.2 g/kg). Dietary digestible lysine in the basal diet was 10.4 g/kg and digestible isoleucine was fixed at 7.1 g/kg (ratio of 68.3 to digestible lysine) since these authors reasoned that the antagonistic effects between leucine and valine were more severe than with isoleucine. The ratio of digestible leucine to digestible lysine ranged from 96 to 173 and for digestible valine from 50 to 108. Increasing dietary digestible leucine resulted in linear (P < 0.001) decreases in feed intake, weight gain and FCE. In contrast, increasing dietary digestible valine resulted in quadratic increases (P < 0.001) in these performance parameters. Additionally, there were significant interactions between digestible leucine × digestible valine for feed intake and weight gain but not for FCE. Interestingly, there was a linear decline in breast meat yield and abdominal lipid with increasing dietary digestible leucine. Furthermore, increasing dietary digestible valine resulted in a quadratic increase (P < 0.001) in thigh yield and a linear decline in abdominal lipid.

The hypothesis that dietary leucine, in excess of standard recommendations, increases protein synthesis and decreases protein degradation in broiler muscle was recently investigated in Ross 308 and Cobb 500 male broilers offered typical, standard 3 phase diets × 3 levels of BCAA to 34 and 35 d post-hatch respectively (Zeit et al., 2019a, 2019b). In the Ross 308 study, the ratio of isoleucine:valine remained constant within each phase over the 3 diets investigated and leucine was incrementally increased by 22%, 15% and 11% in the starter, grower and finisher phases. In the Cobb 500 study, the ratios of leucine:isoleucine:valine were maintained constant within each phase offered whilst, on average, absolute levels were increased by an average 30% between treatments within each phase. In the Ross 308 study, the data indicated that dietary leucine concentrations that exceeded the broiler requirements by as much as 60% neither influenced protein synthesis nor degradation nor muscle growth in growing broilers. Similarly, in the Cobb 500 study, BCAA did not influence protein synthesis or degradation pathways leading to the conclusion that elevated levels do not increase muscle growth at fixed BCAA ratios.

Despite the findings of Zeit et al. (2019a,b) a potential imbalance of BCAA in reduced CP diets should be considered particularly when valine and isoleucine are at minimum dietary levels with correspondingly high leucine levels from maize protein. Whilst antagonism between the BCAA is unlikely to result in depressed broiler performance when practical type diets are offered this may not be the case with reduced-CP diets (Waldroup et al., 2002).
Branched-chain amino acids are actively degraded in extra-hepatic and extra-intestinal tissues and leucine stimulates muscle protein synthesis in mammals. Therefore dietary leucine, isoleucine and valine should be present in an appropriate ratio to prevent amino acids imbalances in meat-type chickens (Wu, 2014).

5.3. Aromatic amino acids: phenylalanine, tyrosine and tryptophan

Phenylalanine is often overlooked by nutritionists as it is seldom limited in conventional broiler diets. However, phenylalanine is an essential amino acid and a precursor for tyrosine. Phenylalanine and tyrosine and tryptophan are aromatic amino acids and phenylalanine influences metabolic processes via its role in the synthesis of thyroid hormones. These hormones have a profound effect on growth performance in broilers by direct effects on oxygen consumption and, in turn, the metabolism of dietary protein, carbohydrates and lipids. Furthermore, they play a crucial role in thermogenesis and adaptation to changes in environmental temperatures (Gropper and Smith, 2012). Of the total phenylalanine plus tyrosine requirement, it was recommended that at least 58% should be supplied in the form of phenylalanine by D’Mello (2003) and similar recommendations of 55% and 57% have been proposed by Rostagno et al. (2011) and Wu (2014), respectively.

The suggested ideal ratio of phenylalanine plus tyrosine to lysine ranged from 95% to 119% in early work (Dean and Scott, 1965; Huston and Scott, 1968; Sasse and Baker, 1972). More recently, ideal ratios of 115% (Dorigam et al., 2013) and 105% (Wu, 2014) have been proposed whilst Franco et al. (2017) suggested a ratio of 113% (10.5 g/kg digestible lysine) to maximise weight gain and breast weight in Cobb 500 male broilers reared from 8 to 17 d post-hatch. These researchers found a significant quadratic response in weight gain when broilers were offered graded levels of phenylalanine plus tyrosine where the regression equation was as follows:

\[
y_{\text{body weight gain}} = 19.21 \times [(\text{Phenylalanine} + \text{Tyrosine})/\text{Lysine}(\%)] - 0.0849 \times [(\text{Phenylalanine} + \text{Tyrosine})/\text{Lysine}(\%)]^2 - 695.14 \quad (r^2 = 0.99).
\]

Decreasing dietary CP from 210 to 165 g/kg in maize-soybean meal diets reduced phenylalanine plus tyrosine from 163% to 117% where phenylalanine provided 57.6% of total in the 165 g/kg CP diet, suggesting that these levels were within the optimum range (Chrystal et al., 2020a). However, in wheat-soybean meal diets with tangible CP reductions there may not be sufficient phenylalanine plus tyrosine. Indeed, in a recent study (unpublished data) in our laboratory, a reduction from 222 to 165 g/kg dietary CP resulted in markedly inferior growth performance in male Ross 308 broilers offered wheat-soybean meal diets from 7 to 35 d post-hatch. The formulated ratio of phenylalanine plus tyrosine to lysine was only 67% suggesting there may have been a deficiency in these 2 amino acids that might partly explain the poor performance observed. The ideal ratio of phenylalanine plus tyrosine to lysine to support optimal growth performance in broilers offered tangibly reduced CP diets warrants further investigation.

6. Peptides

Only a small percentage of proteins are digested by endogenous proteolytic enzymes into individual constituent amino acids and the majority remain as di- and tri-peptides in rats. Thus, a significant amount of the amino acid transport across the gastrointestinal wall occurs as oligopeptides rather than single amino acids (Bai, 1994). Furthermore, absorption of amino acids in a protein-free diet containing only non-bound amino acids has been reported to be less efficient than in diets containing peptides (Gilbert et al., 2008; Ekmay, 2011; Vahdatpour et al., 2016). This is likely because intestinal uptakes of oligopeptides are more rapid than single amino acids (Gilbert et al., 2008). The active transport mechanisms for the transcellular route of peptides include Na⁺ coupled glucose and H⁺ coupled dipeptide transporters and antiporters (Vahdatpour et al., 2016). Transport of di- and tri-peptides takes place primarily via the oligopeptide transporter 1 (PepT1) but also may be absorbed via transcellular movement of cell-penetrating peptides and paracellular movements (Gilbert et al., 2008). A tangible reduction in dietary CP causes fundamental changes to the balance as it decreases di- and tri-peptides derived from ‘intact’ protein but increases single or non-bound amino acids. The relative abundance of chicken PepT1 mRNA expression was investigated in broilers offered diets containing different CP levels by Chen et al. (2005) and a CP × time interaction (P < 0.0001) of PepT1 mRNA expression was observed in broiler chickens reared to 35 d post-hatch. Broilers reared on 120 g/kg CP diets decreased PepT1 mRNA expression whilst it increased in broiler offered 180 and 240 g/kg CP diets. Dietary protein may have a regulatory effect on chicken PepT1 expression at the transcriptional level and peptides, amino acids growth factors and insulin may influence peptide transport through PepT1 in chickens (Chen et al., 2005). Thus, these authors suggest that peptides and amino acids may control expression of PepT1 and conclude that mechanisms by which dietary CP regulates PepT1 gene expression in chickens requires further elucidation. Additionally, studies on the kinetics of glycylproline transport in intestinal brush border vesicles have shown that dipeptides transport is a saturable process and follows Michaelis–Menten kinetics suggesting there may be an appropriate balance between protein-bound and non-bound amino acids for optimum broiler performance (Le Ray et al., 2014; Vahdatpour et al., 2016).

7. Digestive dynamics

Non-bound amino acids do not require digestion, are immediately available for absorption in the small intestine and appear in the portal circulation more rapidly than protein-bound amino acids (Wu, 2009). The extent, rate and site of nutrient digestion should be considered collectively so that glucose and amino acids are made available in appropriately balanced quantities at sites of protein synthesis for efficient growth performance (Liu and Selle, 2015). Digestive dynamics of starch and protein may be described as a 3-tiered process. Firstly, digestion of starch and protein, secondly, absorption of glucose and amino acids from the gut lumen and finally, the transition of these nutrients across enterocytes to enter the portal circulation (Liu and Selle, 2017).

The rate of protein digestion in human nutrition has received attention, particularly with respect to young and elderly subjects (Dangin et al., 2001, 2002; Tang et al., 2009). However, Truong et al. (2017) contended that there should be a greater focus on protein digestion rates for rapidly growing broiler chickens and their application in least-cost feed formulation. Furthermore, non-bound amino acids in broiler diets have inherently different digestive dynamics to protein-bound amino acids (Selle et al., 2015). Individual digestibility coefficients measured at the distal ileum are static, whilst the digestion of starch and protein and subsequent absorption of glucose and amino acids is a dynamic process and is therefore not reflected in these static measurements (Liu and Selle, 2015; Selle et al., 2015). Reducing dietary CP reduces soybean meal content, increases feed grain (and thus starch) and axiomatically increases the content of non-bound amino acids in broiler diets (Selle et al., 2015). Therefore, beyond the supply of adequate dietary amino acids, consideration of digestive dynamics is pivotal to
successful implementation of reduced CP diets utilising substantial quantities of non-bound amino acids.

It has been proposed that rapidly digestible starch could flood the anterior small intestine with glucose to the extent that amino acids compete with glucose co-absorption with sodium and intestinal uptakes via their respective Na⁺-dependent transport systems (Liu and Selle, 2017; Moss et al., 2018a,b). This was unequivocally demonstrated in Moss et al. (2018b) whereby Ross 308 male broilers were offered reduced CP diets (219 to 189 g/kg) accompanied by increased dietary starch (269 to 439 g/kg) using maize starch from 7 to 28 d post-hatch. These authors observed significant (P < 0.05) negative correlations between starch digestibility coefficients and 11 amino acids in the distal ileum. The transition from high to low CP diets increased average starch digestibility coefficients by 23.5% (0.908 vs. 0.735) in proximal jejunum, by 30.3% (0.951 vs. 0.730) in distal jejunum, by 15.9% (0.964 vs. 0.832) in proximal ileum and by 10.9% (0.968 vs. 0.873) in distal ileum. The rate of starch digestion may be important whereby slowly digestible starch may promote catabolism of glucose rather than amino acids in the gut mucosa thereby sparing amino acids and increasing their entry into the portal circulation (Moss et al., 2018b). Thus, in tangibly reduced CP broiler diets with large amounts of supplementary non-bound amino acids, slowly digestible starch may favour absorption of amino acids into the portal circulation.

The importance of starch to protein disappearance rate ratios was demonstrated in Sydenham et al. (2017); Nutrient disappearance rate was calculated as the amount of the nutrient digested or coefficient rate was demonstrated in Sydenham et al. (2017). Nutrient disappearance rate ratio in the order of 3.74:1 would support the best weight gain and FCR outcomes but higher or lower ratios would depress growth performance. That starch digestion rates are more rapid than protein by a factor of 1.70 (4.17 × 10⁻² vs. 2.45 × 10⁻² per min) in the small intestine of birds offered coarsely ground sorghum-soybean meal diets was reported by Liu and Selle (2017) as shown in Fig. 2. Digestion rate describes the slope of the digestion curve in the small intestine by plotting apparent digestibilities in different intestinal segments against their corresponding mean retention time.

Changes in starch, lipid and protein contents as dietary CP is reduced have a profound impact on their digestive dynamics in broiler chickens (Liu et al., 2017). Instructively, the impact of dietary protein on broiler performance was observed to be modified by dietary lipid concentrations and the optimal protein concentration for maximum weight gain was 17.5% lower in birds offered diets with a high lipid concentration. Feed intake was 8.8% less for a diet containing a lipid concentration of 75 g/kg compared with a diet containing 45 g/kg lipid (1,823 vs. 1,999 g/bird). Furthermore, significant (P < 0.001) interactions were reported between dietary protein and lipid on growth performance and nutrient utilisation. Higher dietary lipid inclusions may compromise pellet quality and, in turn, feed intakes in broiler chickens. Another possible explanation for the reduction of feed intake is lipid-induced triggering of the “ileal brake”. Intraluminal infusion of lipids in poultry modulates gastrointestinal motility by increasing duodenal-gastric reflux and reverse peristalsis (Martinez et al., 1995). These researchers suggested that these effects could delay gastric emptying and increase transit time, which is consistent with the “ileal brake”.
mechanism described in mammals (Maljaars et al., 2008). Increased retention time of digesta may increase the extent of nutrient digestion but reduce the rate of nutrient digestion. Very few studies have evaluated the effect of lipid on protein and starch digestion and more studies are required, especially in the context of reduced CP diets. Thus, the impact of dietary lipid and starch in reduced CP diets on digestive dynamics, partitioning of dietary energy and broiler performance needs to be investigated (Liu et al., 2017; Chrystal et al., 2020b).

8. Dietary energy

Iso-energetic diets with reduced CP typically contain more grain, less soybean meal and less added lipid compared with practical diets, inevitably dietary energy derived from starch increases at the expense of energy derived from both dietary lipid and protein whilst relative fat-pad weight and FCR increase as a consequence (Fancher and Jensen, 1989 a, b; Aftab et al., 2006; Siegert et al., 2016; Liu et al., 2017; Chrystal et al., 2020a). The implication is that broilers offered reduced-CP diets over-consume dietary energy relative to protein and deposit this excess as body lipid (Gous et al., 1990). Amino acids are utilised in the gut mucosa primarily for secretory and structural protein synthesis; however, tangible amounts of amino acids undergo catabolism to supply energy to the digestive tract. Approximately 18% of dietary amino acids undergo catabolism in the gut mucosa in pigs (Stoll et al., 1998) but this issue does not appear to have been adequately addressed in poultry. It is possible that non-bound amino acids are less likely to undergo catabolism in the gut mucosa because of their rapid absorption in the anterior small intestine where more starch/glucose is available as an alternative energy substrate (Truong et al., 2017).

The efficiency of utilisation of amino acids for N-corrected metabolizable energy (AME\textsubscript{N}) has been estimated to be 85% for lean gain deposition and 60% for deamination and catabolism. Additionally, the efficiency of utilization of amino acids for net energy (NE) was considered to be 70% for protein deposition and 30% for deamination and catabolism, whilst reported NE values have been determined to be 77.5% of reported AME\textsubscript{N} values (Rostagno et al., 2011; Tillman, 2019). Reducing dietary CP whilst maintaining a minimum ideal amino acid ratio by adding non-bound amino acids is therefore likely to change the efficiency of utilisation of amino acids. Whilst the partitioning of amino acids into lean gain deposition or deamination and catabolism in reduced CP diets requires further elucidation, the concept of “ideal protein” as discussed previously is likely to change as dietary CP is reduced and large amounts of non-bound amino acids are added to these diets. Amino acids absorbed into the portal blood system from the GIT and utilised directly for lean protein deposition in the broiler would not undergo catabolism and would thus not produce associated heat increment of digestion. Thus, in theory, the reduction in heat increment should increase the NE of reduced CP diets and this might partly explain increased lipid deposition in broilers offered these diets. However, in Kamran et al. (2008) broilers offered maize-soybean-based diets from 1 to 35 d post-hatch with graded reductions in CP from 200 to 170 g/kg in which the energy-to-protein ratio was maintained, resulted in depressed weight gains of 13.4% (610 vs. 704 g/bird) and compromised FCE by 29.2% (337 vs. 476 g/kg). More recently, a reduction in dietary AME\textsubscript{N} from 12.01 MJ/kg in tangibly reduced CP (143 g/kg) maize-soybean meal-based diets resulted in an 18.5% decrease (P < 0.001) in relative fat-pad weights (10.1 vs. 12.4 g/kg) but weight gain and FCR were inferior (1,760 vs. 1,879 g/bird; P = 0.024 and 1,765 vs. 1,629 g/g; P < 0.001) in male Ross 308 broilers from 14 to 35 d post hatch (Chrystal et al., 2020b). Thus, further research on energy metabolism and body lipid deposition is warranted for tangibly reduced CP diets. The associated changes in energy-supplying substrate, competition for absorption sites and the ratios of dietary starch to protein, in conjunction with the rate of digestion of these substrates will all need to be considered when dietary CP is reduced and large amounts of non-bound amino acids are added (Liu and Selle, 2015). Changes in nutrient utilisation as a function of dietary lipid, starch and protein whilst enhancing digestive tract function with consideration of dietary fibre, feed particle size, pellet durability, pellet hardness and whole grain feeding may advantage tangibly reduced CP diets by changing underlying digestion dynamics and energy metabolism in broilers.

9. Gizzard function, particle size, fibre and whole grain

Dietary fibre, feed particle size and whole grain have been reported to enhance gizzard function and improve nutrient availability for feed for broilers. Inclusion of whole grain or coarse fibre at 30 g/kg or maintaining coarse-ground cereal (≥1.0 mm) at least 200 to 300 g/kg in broiler feed achieves heavier gizzards and enhances gizzard function (Svihus, 2011; Mateos et al., 2012; Abdollahi et al., 2018; Kheravii et al., 2018). Furthermore, Svihus (2011) noted that gizzards responded very rapidly to changes in dietary composition. Enhanced gizzard function using whole grain has been demonstrated to improve FCE in broilers (Singh et al., 2014; Liu et al., 2015; Moss et al., 2017; Abdollahi et al., 2018). Multi-factorial mechanisms underlying nutrient utilisation, improved digestibility and broiler performance through manipulation of dietary structural components have been proposed by Kheravii et al. (2018). However, addition of whole maize at 250 g/kg to reduced-CP maize-soybean-based diets had no effect on gizzard size or pH and was unable to ameliorate the effects of reduced CP (165 g/kg) on broiler performance parameters of Ross 308 off-sex male broilers from 14 to 35 d post-hatch (Chrystal et al., 2020a). Similarly in wheat-soybean-based diets, the addition of 250 g/kg whole wheat post pelleting had no impact on the performance of Ross 308 off-sex male broilers offered reduced CP (165 g/kg) diets from 14 to 35 d post-hatch, although relative gizzard weights increased (P < 0.001) by 53.8% (13.41 vs. 8.72 g/kg) gizzard content pH reduced (2.76 vs. 3.62; P < 0.001) and relative abdominal fat pad weights decreased (P < 0.05) by 14.6% (6.91 vs. 8.09 g/kg) (Yin et al., 2020).

10. Exogenous feed enzymes

Reductions in dietary CP are usually achieved by increasing the feed grain content at the expense of protein meal (soybean meal) with concomitant increases in dietary starch and reductions in dietary lipid. Since exogenous feed enzymes are reported to be substrate dependant, exogenous enzymes capable of degrading non-starch polysaccharides in broiler diets based on “viscous” grains, including wheat and barley, have been widely adopted (Selle and Ravindran, 2007). Thus, by implication, the contribution of non-starch polysaccharide enzymes such as xylanase in wheat-based, reduced CP diets may confound results and therefore they are not normally included in these trials. However, this also limits useful, direct comparisons of trial data with standard commercial broiler diets.

Perhaps exogenous phytase is even more relevant since it goes beyond releasing phytate-bound phosphorus with “extra-phosphor effects” on protein/amine acids, calcium and sodium (Shelton et al., 2004) and therefore results in even greater confounding effects in reduced CP diets with changing dietary phytate levels. Phytate has the potential to form binary or ternary protein-phytate complexes depending on the environmental pH and the isoelectric point of the protein body (Selle et al., 2000) and Kidd...
et al. (2016) explained the interactions between phytate and threonine and the formation of phytate–lysinic complexes. Consequently, amino acid digestibilities were compromised in diets without phytase supplementation (Selle et al., 2000). Unsurprisingly therefore, most studies with reduced CP diets do not include exogenous phytase even though it is standard practice to do so in commercial broiler diets, often at levels providing 1,500 FTU/kg or more. Additionally, dietary phytate and phytase influence the digestion dynamics of starch/glucose and protein/amino acids and exogenous phytase is likely to have a positive bearing on the post-enteral bioavailability of glucose and amino acids via its influence on Na⁺-dependent intestinal uptake of these nutrients. Furthermore, it has been suggested that phytate and phytase may have reciprocal impacts on Na⁺, K⁺-ATPase activity adding further complexity to the successful development of tangibly reduced CP diets (Moss et al., 2018a). The inevitable resulting conundrum on including exogenous phytase in reduced CP diets is the allocation of suitable matrix values to the phytase as dietary phytate changes and substantial amounts of supplemental, non-bound amino acids are included. Whilst it is prudent to allocate only available phosphorus, calcium and sodium matrix values to phytase in tangibly reduced CP diets, the extra-phosphoric effects cannot be ignored.

11. Dietary electrolyte balance

Dietary electrolyte balance (DEB) may be calculated from the following equation (Mongin, 1981):

\[
\text{DEB (mEq/kg)} = \frac{\text{Na}^+ (\text{mg/kg}) \times 23.0 + \text{K}^+ (\text{mg/kg}) \times 39.1 - \text{Cl}^- (\text{mg/kg}) \times 35.5}{
\]

DEB plays an influential role in the homeostasis of body fluids and in maintaining the acid-base balance. A DEB of approximately 250 mEq/kg would appear to be optimal although a wide range of acceptable DEB has been reported in the literature; from 200 to 350 mEq/kg, depending on environmental temperature, humidity and other factors (Borges et al., 2011).

Much of the research into reduced-CP diets has overlooked DEB. As dietary CP is reduced so too is DEB and Waldroup (2007) suggested that these lower DEB values may be contributing to the poor performance of birds offered reduced-CP diets. This possibility was specifically investigated in Chrystal et al. (2020b). A comparison was drawn between 156 g/kg CP broiler diets with a DEB of either 230 or 120 mEq/kg. However, this difference in DEB did not have any significant influence on growth performance or fat deposition in Ross 308 off-sex male broilers from 14 to 35 d post-hatch. Nevertheless, it may be prudent to maintain DEB in reduced-CP diets rather than allowing it to decline.

12. Conclusions

Multiple factors that include both amino acid and non-amino acid limitations with consideration of digestion dynamics will need to be considered for the successful development of reduced CP diets. Conditionally essential amino acids such as Gln equivalent together with threonine and choline, aromatic amino acids (phenylalanine and tyrosine) with consideration of amino acid antagonisms (lysine & arginine; BCAA) and possible changes in the ratios of amino acids to lysine with reduced CP diets require further elucidation.

The efficiency of absorption of amino acids and peptides and the competition between amino acids and glucose for Na⁺-dependent transporters will need to be considered whilst the fate of ingested non-bound amino acids need to be addressed. Manipulation of the ratio of starch:protein disappearance rates in the proximal small intestine may be a key factor in optimising the availability of amino acids, peptides and glucose along the gastro-intestinal tract. Finally, the ultimate solution to the successful implementation of tangibly reduced CP diets, utilising substantial amounts of non-bound amino acids, will be the control of excess body lipid deposition in meat-type poultry offered these diets, whilst maintaining growth rates and FCR.

Conflict of interest

We declare that we have no financial and personal relationships with other people or organizations that can inappropriately influence our work, there is no professional or other personal interest of any nature or kind in any product, service and/or company that could be construed as influencing the content of this paper.

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