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Original Research Article

# Implications of excreta uric acid concentrations in broilers offered reduced-crude protein diets and dietary glycine requirements for uric acid synthesis

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## ABSTRACT

In a previous experiment, male Ross 308 broiler chickens were offered dietary treatments with 3 levels of crude protein (222, 193, 165 g/kg) and 3 feed grains (ground maize, ground wheat, whole wheat) from 7 to 35 d post−hatch. Maize-based diets supported superior growth performance in comparison to wheat-based diets. Uric acid concentrations in excreta were retrospectively determined and related to total nitrogen (N) excreta concentrations. Uric acid concentrations ranged from 28.5 to 69.4 mg/g and proportions of uric acid-N to total excreta-N ranged from 27.4% to 42.6% in broiler chickens offered the 3 × 3 factorial array of dietary treatments. Proportions of uric acid-N to total N in excreta in birds offered the 165 g/kg CP, maize-based diet were significantly lower by 10.6 percentage units (27.4% versus 38.0%; $P = 0.00057$) than their wheat-based counterparts. Total excreta analysed had been collected from 35 to 37 d post−hatch when feed intakes and excreta outputs were monitored. There were linear relationships between proportions of uric acid-N to total N in excreta in birds offered the three 165 g/kg CP diets with weight gain ($r = -0.587$; $P = 0.010$), feed intake ($r = -0.526$; $P = 0.025$) and feed conversion ratios ($r = 0.635$; $P = 0.005$). The possibility that increasing uric acid-N proportions in excreta is indicative of excessive ammonia accumulations compromising growth performance is discussed. The mean proportion of dietary glycine involved in uric acid excretion was 49.2% across all dietary treatments but ranged from 25.0% to 80.9%. Thus, the appropriate amount of dietary glycine is variable and largely dependent on the volume of uric acid synthesised and excreted.

## 1. Introduction

Decades ago, Miles and Featherstone, (1976) argued that uric acid excretion could serve as a gauge of protein quality in broiler diets. However, total nitrogen (N) in avian excreta is derived from a combination of uric acid in urine and undigested protein in faeces (Kroghahl and Dalsgard, 1981), which is a complicating factor. Moreover, the percentage of dietary N excreted as uric acid will increase as dietary N concentrations increase as Creek and Vasaitis (1961) reported a 2-fold increase, from 14.3% to 28.0%, in 12-d-old

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birds when dietary protein levels were increased from 0.50 to 1.25 g/kg of adequacy. Ammonia N ($NH_3$—N) is ultimately excreted as uric acid; therefore, any increases in $NH_3$ will increase uric acid in excreta. Instructively, Snetsinger and Scott, (1961) reported that inferior broiler growth performance caused by dietary amino acid imbalances was partially alleviated by glycine, which was attributed to the requisite involvement of this amino acid in the Krebs uric acid cycle to generate uric acid in excreta. Relatively recently, Donsbough et al., (2010) contended that uric acid concentrations in avian excreta may be taken as an indicator of the efficiency of amino acid utilisation by broiler chickens offered diets with either adequate or inadequate crude protein (CP) levels. Given this, uric acid concentrations in excreta from the Chrystal et al., (2021) study were determined retrospectively because uric acid concentrations could prove instructive to the interpretation of the unequivocal outcomes reported.

The hypothesis in Chrystal et al., (2021) that maize is superior to wheat as the feed grain basis of reduced-CP diets was established as maize clearly supported superior broiler growth performance compared to wheat when it was the feed grain basis of 165 g/kg reduced-CP diets. However, the performance of birds offered reduced-CP, wheat-based diets was remarkably substandard. The tentative premise developed was that the inferior performance of birds offered reduced-CP, wheat-based diets was due to 'ammonia overload' or an accumulation of excessive $NH_3$ levels arising from amino acid imbalances and the deamination of surplus amino acids (Selle et al., 2020). The determination of systemic plasma $NH_3$ concentrations is quite straightforward; however, their validity is challenged by their volatility over time (Okumura and Tasaki, 1969). Thus, the time taken to draw blood samples from a given number of birds may be sufficient to confound the assays. At this institution, Moss et al. (2019) determined $NH_3$ concentrations in plasma taken from the anterior mesenteric vein but it was found that $NH_3$ concentrations increased quadratically ($r = 0.703$; $P < 0.001$) with time elapsed to take blood samples irrespective of treatments. Therefore, in lieu of plasma $NH_3$, excreta samples from the Chrystal et al. (2021) study were analysed retrospectively for uric acid concentrations, so that concentrations of uric acid—N in excreta, relative to total-N dietary intakes, could be assessed. $NH_3$—N arising from the deamination surplus amino acids is ultimately excreted as uric acid-N; therefore, absolute concentrations of uric acid in excreta and/or uric acid—N excreta outputs as proportions of total-N dietary intakes might be indicative of excessive $NH_3$ concentrations in systemic plasma.

Wheat is the dominant feed grain in diets for broiler chickens in Australia, sorghum is second, but maize is rarely used. It is against this background that reduced-crude protein (CP) diets are being developed locally because of the potential they hold for sustainable chicken-meat production (Greenhalgh et al., 2020a). Birds were offered 215 and 165 g/kg CP wheat-based diets, without and with whole grain, from 14 to 35 d post—hatch in Yin et al. (2020). Weight gain and feed intake were not compromised by reduced-CP diets with a numerical increase of 3.36% (3,197 versus 3,093 g/bird; $P = 0.058$) in feed intake, which closely approached significance. However, FCR of birds offered reduced-CP, wheat-based diets was compromised by 5.99% (1.576 versus 1.487) in comparison to standard CP diets. In contrast, growth performance of birds offered 180 and 162.5 g/kg CP, wheat-based diets was remarkably inferior in Greenhalgh et al. (2020b) and on a similar scale to Chrystal et al. (2021). Thus, the successful development of reduced-CP diets based on wheat is a challenge but determinations of uric acid concentrations in excreta could prove instructive. Thus, the purpose of this paper is to report the retrospectively determined uric acid excreta concentrations from Chrystal et al. (2021) and to discuss their implications.

## 2. Materials and methods

The methodology employed in the original feeding study is detailed in Chrystal et al. (2021) and the study fully complied with guidelines (2019/1497) approved by the Research Integrity and Ethics Administration of The University of Sydney. Essentially, the experimental design was a 3 × 3 factorial array of dietary treatments with 3 levels of dietary CP (222, 193 and 165 g/kg) and 33 feed grains: maize, ground wheat, whole wheat. Whole wheat was incorporated into the ration at 150 g/kg post-pelleting from 14 to 35 d post—hatch and the dietary treatments were offered to a total of 324 birds from 7 to 35 d post—hatch. Total excreta were collected and feed intakes monitored for each replicate cage from 32 to 34 d post—hatch to determine parameters of nutrient utilisation in the Chrystal et al. (2021) study.

The excreta from the total collection period were retrospectively analysed for uric acid concentrations using reversed-phase (RP) HPLC with isocratic elution and UV detection. The procedure was based on the method of Markelj et al. (2016) but optimised for chicken excreta samples. In brief, finely-ground excreta samples were freshly prepared in duplicate by incubating for 1hr in 0.1 mol/L glycine, pH 9.7 at 37 °C, with mixing. Excreta extracts were centrifuged for 5 min at 2,000 × *g* and the supernatant collected, diluted in acetate running buffer (50 mmol/L sodium acetate, 1% ACN, pH 4.0), centrifuged and filtered. Chromatography was performed on an Agilent 1290 Infinity HPLC system, using a Zorbax Eclipse XDB column, 4.6 × 250 mm, 5 μm (Agilent, 993967-902) thermostatted at 35 °C. Chromatography was performed isocratically, with the above acetate buffer as the mobile phase, run at 1.0 mL/min, with UV detection at 285 nm. All reagents including uric acid were purchased from Sigma—Aldrich. Prior to performing the assay, the method was validated and shown to successfully resolve uric acid from other purine metabolites such as allantoin, creatine, hypoxanthine and xanthine (data not shown). A standard curve was generated using freshly prepared uric acid standards in 0.1 mol/L glycine, pH 9.7.

The molecular weight of uric acid ($C_5H_4N_40_3$) is 168.11 Da and it contains 333 g/kg N and the molecular weight of glycine ($C_2H_5N0_2$) is 75.07 Da. These values were used to calculate the proportion of uric acid-N to total-N in excreta, the proportion of excreta uric acid-N to dietary N intake and the proportion of dietary glycine intake utilised for uric acid synthesis. The experimental data were subject to analyses of variance using the SPSS Statistics 24 program (IBM Corporation. Somers, NY USA). Pearson correlations, linear and quadratic regressions and were examined when appropriate and the significance of pair-wise comparisons was considered when relevant. Experimental units were cage means (6 replicate cages of 6 birds per dietary treatment) and a probability level of less than 5% by Student's *t*-test was considered statistically significant.

## 3. Results

The effects of dietary treatments on absolute concentrations of total-N and uric acid in excreta and the proportion of uric acid-N to total-N in excreta are shown in Table 1, where significant treatment interactions between feed grain type and dietary CP levels were observed for the 3 parameters. Concentrations of total-N in excreta declined by 41.2% (33.8 versus 57.5 mg/g) following the transition from 222 to 165 g/kg CP diets in birds offered maize-based diets. However, the corresponding falls in excreta total-N concentrations, while still significant, were more moderate with similar declines of 19.9% (30.6 versus 38.2 mg/g) and 22.3% (33.4 versus 43.0 mg/g) in birds offered ground wheat and whole wheat diets, respectively. Uric acid concentrations in excreta also declined with the 222 to 165 g/kg CP diet transition. In birds offered maize-based diets, uric

**Table 1**
Effects of dietary treatments on absolute concentrations of total nitrogen (N) and uric acid in excreta and proportion of uric acid-N to total N in excreta.

| Treatment | | Excreta-N, mg/g | Uric acid, mg/g | Proportion of uric acid-N to excreta-N, % |
|---|---|---|---|---|
| Feed grain | Crude protein, g/kg | | | |
| Maize | 222 | $57.5^{e}$ | $69.4^{e}$ | $40.0^{de}$ |
| (ground) | 193 | $39.2^{cd}$ | $42.6^{bc}$ | $35.3^{bc}$ |
| | 165 | $33.8^{abc}$ | $28.5^{a}$ | $27.4^{a}$ |
| Wheat | 222 | $38.2^{bcd}$ | $43.9^{c}$ | $37.1^{cde}$ |
| (ground) | 193 | $33.6^{ab}$ | $34.5^{ab}$ | $33.6^{bc}$ |
| | 165 | $30.6^{a}$ | $35.5^{ab}$ | $38.0^{cde}$ |
| Wheat | 222 | $43.0^{d}$ | $54.2^{d}$ | $42.6^{e}$ |
| (whole) | 193 | $36.0^{abc}$ | $36.6^{abc}$ | $33.3^{bc}$ |
| | 165 | $33.4^{ab}$ | $30.5^{a}$ | $29.9^{ab}$ |
| SEM | | 1.945 | 2.860 | 2.017 |
| Main effect: feed grain | | | | |
| Maize | | 43.5 | 46.8 | 34.2 |
| Wheat (ground) | | 34.1 | 38.0 | 36.2 |
| Wheat (whole) | | 37.1 | 40.4 | 35.3 |
| Crude protein, g/kg | | | | |
| 222 | | 45.9 | 55.8 | 39.9 |
| 193 | | 36.3 | 37.9 | 34.1 |
| 165 | | 32.6 | 31.5 | 31.8 |
| Significance (*P*-value) | | | | |
| Feed grain (FG) | | <0.001 | 0.001 | 0.488 |
| Crude protein (CP) | | <0.001 | <0.001 | <0.001 |
| FG × CP interaction | | <0.001 | <0.001 | 0.004 |

[a to e] Means within columns not sharing a common superscript are significantly different at the 5% level of probability.

**Table 2**
Effects of dietary treatments on quantities of dietary N intakes, uric acid-N outputs and the proportion of uric acid-N to dietary N over the total excreta collection period from 35 to 37 d post–hatch and dietary glycine intakes and proportion of dietary glycine utilised for uric acid synthesis.

| Treatment | | Dietary N intake, g/cage | Uric acid-N Output, g/cage | Proportion of uric acid-N to dietary-N, % | Glycine intake, g/cage | Glycine proportion of intake for uric acid synthesis, % |
|---|---|---|---|---|---|---|
| Feed grain | Crude protein, g/kg | | | | | |
| Maize | 222 | 65.5 | $11.4^{e}$ | $17.7^{e}$ | $19.1^{bc}$ | $80.9^{e}$ |
| (ground) | 193 | 56.0 | $5.6^{c}$ | $10.2^{bc}$ | $17.2^{ab}$ | $45.0^{bc}$ |
| | 165 | 49.9 | $3.3^{a}$ | $6.9^{a}$ | $18.8^{bc}$ | $25.0^{a}$ |
| Wheat | 222 | 69.2 | $9.4^{d}$ | $13.9^{d}$ | $20.1^{bc}$ | $59.3^{d}$ |
| (ground) | 193 | 63.9 | $6.3^{c}$ | $9.9^{bc}$ | $20.3^{c}$ | $42.3^{bc}$ |
| | 165 | 43.5 | $5.3^{bc}$ | $12.3^{cd}$ | $15.8^{a}$ | $45.9^{c}$ |
| Wheat | 222 | 71.1 | $9.8^{de}$ | $13.9^{d}$ | $20.9^{c}$ | $64.6^{d}$ |
| (whole) | 193 | 57.2 | $5.6^{c}$ | $9.8^{bc}$ | $17.3^{ab}$ | $44.4^{bc}$ |
| | 165 | 44.1 | $3.9^{ab}$ | $8.8^{ab}$ | $14.9^{a}$ | $35.5^{b}$ |
| SEM | | 3.230 | 0.564 | 0.973 | 1.041 | 1.041 |
| Main effect: feed grain | | | | | | |
| Maize | | 57.1 | 6.8 | 11.6 | 18.4 | 18.4 |
| Wheat (ground) | | 58.9 | 7.0 | 12.0 | 18.8 | 18.8 |
| Wheat (whole) | | 57.5 | 6.5 | 10.8 | 17.7 | 17.7 |
| Crude protein, g/kg | | | | | | |
| 222 | | $68.6^{c}$ | 10.5 | 15.1 | 20.0 | 20.0 |
| 193 | | $59.0^{b}$ | 5.8 | 9.9 | 18.3 | 18.3 |
| 165 | | $45.8^{a}$ | 4.2 | 9.3 | 16.5 | 16.5 |
| Significance (*P*-value) | | | | | | |
| Feed grain (FG) | | 0.872 | 0.510 | 0.345 | 0.456 | 0.456 |
| Crude protein (CP) | | <0.001 | <0.001 | <0.001 | 0.001 | 0.001 |
| FG × CP interaction | | 0.158 | 0.019 | 0.001 | 0.019 | 0.019 |

[a to e] Means within columns not sharing a common superscript are significantly different at the 5% level of probability.

acid concentrations fell by 58.9% (28.5 versus 69.4 mg/g), by 19.1% (33.5 versus 43.9 mg/g) in birds offered ground wheat diets and by 43.7% (30.5 versus 54.2 mg/g) in birds offered whole wheat diets. The lowest proportion of uric acid-N to total-N in excreta of 27.4% was recorded for birds offered 165 g/kg CP maize-based diets. This was significantly lower by 10.6 percentage units (27.4% versus 38.0%; $P = 0.00057$) than in birds offered 165 g/kg CP ground wheat-based diets, where the probability value is based on a pair-wise comparison. However, there was not a difference (27.4% versus 29.9%) between the 165 g/kg maize and whole wheat diets. In contrast, there were no significant effects of dietary CP levels on uric acid-N proportions of excreta total-N in excreta in birds offered ground wheat diets.

The effects of dietary treatments on quantities of dietary N intakes, uric acid-N outputs and the proportion of uric acid-N to dietary N over the total excreta collection period from 35 to 37 d post–hatch are shown in Table 2. Also, dietary glycine intakes and proportion of dietary glycine utilised for uric acid synthesis are tabulated. Predictably, dietary N intakes declined ($P < 0.001$) with reductions in dietary CP from 68.6 to 59.0 and 57.5 g per replicate cage. Similarly, outputs of uric acid-N declined ($P < 0.001$) from 10.5 to 5.8 and 4.2 g per replicate cage but there was a treatment interaction ($P = 0.019$). When the 222 and 165 g/kg CP diets are compared, uric acid-N outputs fell by 43.6% for ground wheat diets, 60.2% for whole wheat diets but by 71.1% for maize-based diets. There was a treatment interaction ($P = 0.001$) for the proportion of excreted uric acid-N to dietary-N where the proportion significantly declined from 17.7% to 6.9% with the transition from 222 to 165 g/kg CP in maize-based diets. In contrast, with ground wheat diets the same CP transition did not alter proportions (12.3% versus 13.9%) but there was a significant reduction in whole wheat diets from 13.9 to 8.8 g uric acid-N per replicate cage. There was a treatment interaction ($P = 0.019$) for glycine intake; the glycine intakes did not vary in birds offered maize-based diets with an average intake of 18.4 g per cage. However, following the transition from 222 to 165 g/kg CP diets, glycine intakes declined by 21.4% (15.8 versus 20.1 g/cage) for ground wheat diets and by 28.7% (14.9 versus 20.9 g/cage) for whole wheat diets. This difference essentially stemmed from lower feed intakes during the total collection period as analysed dietary glycine concentrations were similar. Proportions of dietary glycine intakes required for uric acid synthesis were subject to a treatment interaction ($P = 0.019$). This proportion declined by 55.9 percentage units (25.0% versus 80.9%) following the transition from 222 to 165 g/kg CP in maize-based diets but the corresponding decline in ground wheat diets was 13.4 percentage units (45.9% versus 59.3%) and in whole wheat diets it was 29.1 percentage units (35.5% versus 64.6%).

The effects of dietary treatments on concentrations of free amino acids in systemic plasma at 34 d post–hatch are shown in Table 3. This data was partially presented in Chrystal et al. (2021) but it is reproduced here as it is relevant. The amino acids were selected because of their involvement in the condensation of ammonia and glutamic acid to glutamine (Minet et al., 1997), the involvement of glycine in the Krebs uric acid cycle (Salway, 2018) and that serine (Meléndez-Hevia and de Paz-Lugo, 2008) and threonine (Baker et al., 1972) can serve as precursors for glycine biosynthesis. A treatment interaction ($P < 0.001$) was observed for threonine. Following the transition from 222 to 165 g/kg CP diets, free threonine concentrations increased by 39.7% (102.8 versus 73.6 μg/mL) in birds offered maize-based diets and by 29.6% (108.2 versus 83.5 μg/mL) with whole wheat diets but by 100.4% (179.6 versus 89.6 μg/mL) with ground wheat diets. Treatment effects on glycine, serine and glycine equivalents followed similar patterns. Concentrations of these amino acids were significantly higher with the two sets of wheat-based diets than maize-based diets and plasma concentrations significantly increased as dietary CP levels declined. Glutamate and glutamine are considered collectively but glutamine made up the majority (89.5%) of the total, overall. Concentrations of glutamine plus glutamate were significantly higher in birds offered wheat-based diets than maize and concentrations increased significantly when dietary protein was reduced to less than 222 g/kg CP. Total free amino acid concentrations followed a similar pattern. On average, birds offered wheat-based diets had significantly higher concentrations by 13.3% (835 versus 737 μg/mL) than their counterparts offered maize-based diets. The transition from 222 to 165 g/kg CP diets significantly increased total free amino acid concentrations by 9.82% (850 versus 774 μg/mL).

## 4. Discussion

In the previously reported Chrystal et al. (2021) study, broiler chickens were offered either maize–or two sets of wheat-based diets containing 222, 193 and 165 g/kg crude protein (CP) where one wheat-based diet contained 150 g/kg whole wheat. The performance of birds offered the standard 222 g/kg CP wheat-based diet was significantly better than the corresponding maize-based diet in weight gain with an improvement of 8.54% (2,403 versus 2,214 g/bird) plus an 8.70% higher feed intake (3,487 versus 3,208 g/bird). However, birds offered the 165 g/kg CP maize-based diet outperformed their ground wheat-based counterparts in weight gain by 53.0% (2370 versus 1549 g/bird), in feed intake by 22.4% (3,481 versus 2,843 g/kg) and in FCR by 19.9% (1.473 versus 1.840)

**Table 3**
Effects of dietary treatments on concentrations of selected free amino acids in systemic plasma at 34 d post–hatch.

| Treatment | | Threonine, μg/mL | Glycine, μg/mL | Serine, μg/mL | Glycine equivalents[1], μg/mL | Glutamate plus glutamine, μg/mL | Total amino acids, μg/mL |
|---|---|---|---|---|---|---|---|
| Feed grain | Crude protein, g/kg | | | | | | |
| Maize (ground) | 222 | $73.6^{a}$ | 46.1 | 56.3 | 86.3 | 139.5 | 696 |
| | 193 | $77.2^{a}$ | 52.6 | 57.3 | 93.5 | 180.6 | 753 |
| | 165 | $102.8^{cd}$ | 56.5 | 64.4 | 102.5 | 192.5 | 763 |
| Wheat (ground) | 222 | $89.6^{abcd}$ | 50.2 | 63.4 | 95.5 | 183.4 | 803 |
| | 193 | $99.1^{bcd}$ | 50.7 | 63.2 | 95.9 | 213.8 | 819 |
| | 165 | $179.6^{e}$ | 66.8 | 78.6 | 123.0 | 190.2 | 935 |
| Wheat (whole) | 222 | $83.5^{abc}$ | 51.5 | 66.1 | 98.7 | 199.4 | 824 |
| | 193 | $79.3^{ab}$ | 51.9 | 66.6 | 99.5 | 217.3 | 775 |
| | 165 | $108.2^{d}$ | 66.6 | 81.8 | 125.0 | 193.3 | 854 |
| SEM | | 7.431 | 2.325 | 2.785 | 3.809 | 10.072 | 36.23 |
| Main effect: feed grain | | | | | | | |
| Maize | | 84.5 | $51.7^{a}$ | $59.3^{a}$ | $94.1^{a}$ | $170.8^{a}$ | $737^{a}$ |
| Wheat (ground) | | 122.8 | $55.9^{b}$ | $68.4^{b}$ | $104.8^{b}$ | $195.8^{b}$ | $852^{b}$ |
| Wheat (whole) | | 90.3 | $56.7^{b}$ | $71.5^{b}$ | $107.7^{b}$ | $203.6^{b}$ | $818^{b}$ |
| Crude protein, g/kg | | | | | | | |
| 222 | | 82.2 | $49.3^{a}$ | $61.9^{a}$ | $93.5^{a}$ | $174.1^{a}$ | $774^{a}$ |
| 193 | | 85.2 | $51.7^{a}$ | $62.4^{a}$ | $96.3^{a}$ | $203.9^{b}$ | $782^{a}$ |
| 165 | | 130.2 | $63.3^{b}$ | $75.0^{b}$ | $116.8^{b}$ | $192.1^{b}$ | $850^{b}$ |
| Significance (P-value) | | | | | | | |
| Feed grain (FG) | | <0.001 | 0.026 | <0.001 | <0.001 | 0.001 | 0.001 |
| Crude protein (CP) | | <0.001 | <0.001 | <0.001 | <0.001 | 0.003 | 0.025 |
| FG × CP interaction | | <0.001 | 0.099 | 0.487 | 0.152 | 0.058 | 0.381 |

[a to d] Means within columns not sharing a common superscript are signif1icantly different at the 5% level of probability.

[1] Glycine equivalents = glycine + (serine × 0.7143).

**Table 4**
Effects of dietary treatments on growth performance from 7 to 35 d post—hatch analysed as a 3 × 2 factorial array with 3 feed grains (maize, wheat-ground, wheat-whole) and either standard (222 g/kg) or reduced (165 g/kg) dietary crude protein concentrations (adapted from Chrystal et al., 2021).

| Treatment | | Growth performance | | |
|---|---|---|---|---|
| Feed grain | Crude protein, g/kg | Weight gain, g/bird | Feed intake, g/bird | FCR, g/g |
| Maize | 222 | 2,214$^{b}$ | 3,208$^{b}$ | 1.453$^{b}$ |
| | 165 | 2,370$^{bc}$ | 3,481$^{c}$ | 1.473$^{b}$ |
| Wheat | 222 | 2,403$^{c}$ | 3,487$^{c}$ | 1.453$^{b}$ |
| (ground) | 165 | 1,549$^{a}$ | 2,843$^{a}$ | 1.840$^{a}$ |
| Wheat | 222 | 2,436$^{c}$ | 3,427$^{c}$ | 1.407$^{b}$ |
| (whole) | 165 | 1,498$^{a}$ | 2,685$^{a}$ | 1.798$^{a}$ |
| SEM | | 55.60 | 71.83 | 0.0378 |
| Main effect: feed grain | | | | |
| Maize | | 2,292 | 3,344 | 1.463 |
| Wheat (ground) | | 1,978 | 3,165 | 1.646 |
| Wheat (whole) | | 1,967 | 3,056 | 1.603 |
| Crude protein, g/kg | | | | |
| 222 | | 2,351 | 3,374 | 1.438 |
| 165 | | 1,805 | 3,003 | 1.704 |
| Significance (*P*-value) | | | | |
| Feed grain (FG) | | <0.001 | 0.001 | <0.001 |
| Crude protein (CP) | | <0.001 | <0.001 | <0.001 |
| FG × CP interaction | | <0.001 | <0.001 | <0.001 |

[a, b, c] Means within columns not sharing a common superscript are signif1icantly different at the 5% level of probability.

from 7 to 35 d post—hatch. These remarkable differences in growth performance are detailed in Table 4 in which data for the intermediate 193 g/kg CP diets have been omitted in the interests of clarity.

As evident in Table 4, the transition from 222 to 165 g/kg CP maize-based diets was accommodated by broiler chickens; in contrast, the same CP transition in the two wheat-based diets seriously compromised bird performance to similar extents. It has been suggested that this could be a consequence of, effectively, $NH_3$ toxicity or 'ammonia overload' (Selle et al., 2020). As stated, total N in avian excreta is derived from both undigested protein in faeces and uric acid in urine (Kroghahl and Dalsgard, 1981). Thus, the decline in excreta-N with reductions in dietary CP observed in the present study (Table 1) can be mainly attributed to less undigested protein in excreta. Uric acid concentrations in excreta also declined with reductions in dietary CP although it is noteworthy that numerically less uric acid was excreted by birds offered maize-based diets in comparison to ground wheat and whole wheat diets by 19.7% and 6.56%, respectively. However, when uric acid-N is expressed as a proportion of total dietary N intake, there was a significant reduction of 10.6 percentage units (27.4% versus 38.0%) when the 165 g/kg CP maize and ground wheat diets are compared. This was a highly significant ($P < 0.001$) reduction based on a pair-wise comparison.

The kinetic data in Table 2 is probably more meaningful. In the corresponding comparison, the reduction in proportion of uric acid-N output to dietary N intake was 5.4 percentage units (6.9% versus 12.3%; $P < 0.001$) over the total excreta collection period, which again was highly significant. Also, there was a numerical reduction of 21.6% or 1.9 percentage units (6.9% versus 8.8%) when the 165 g/kg CP maize and whole wheat diets were compared. The likelihood is that these relative increases in uric acid excretion may be attributed to greater extents of amino acid deamination in wheat-based diets, especially ground wheat diets, which are the more valid comparison. As illustrated in Fig. 1, there is a linear relationship ($r = -0.587$; $P = 0.010$) between proportions of uric acid-N output to dietary N intake and weight gain in birds offered the three 165 g/kg CP diets. There are also linear relationships between these proportions with feed intake ($r = -0.526$; $P = 0.025$) and FCR ($r = 0.635$; $P = 0.005$) as shown in Figs. 2 and 3, respectively. These linear relationships are not conclusive, but they are consistent with the premise that hyperammonaemia, or ammonia overload, as reflected by uric acid-N outputs in relation to dietary N intakes, is adversely influencing bird growth performance when reduced-CP wheat-based diets are offered.

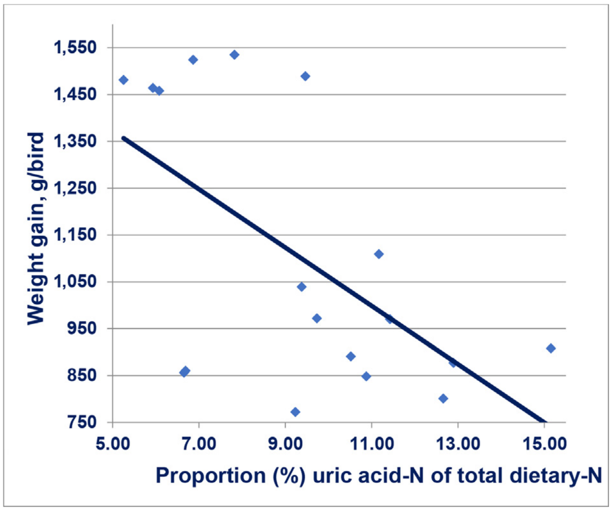

**Fig. 1.** Linear relationship ($r = -0.587$; $P = 0.010$) between proportion of uric acid-N output in excreta of dietary total-N input over the total collection period in birds offered three 165 g/kg CP diets and weight gain, where Weight gain = 1,684 − 62.3 × Proportion.

High blood $NH_3$ levels have been shown to depress feed intakes in rats offered amino acid imbalanced diets via mechanisms involving the central nervous system (Noda, 1975; Noda and Chikamori, 1976). Moreover, in poultry, high circulating $NH_3$ levels have been reported to depress broiler growth performance by Namroud et al. (2008) and Ospina-Rojas et al. (2013, 2014). Alternatively, elevated uric acid concentrations in systemic plasma were associated with depressed feed intakes in broiler chickens from 22 to 42 d in Sigolo et al. (2017).

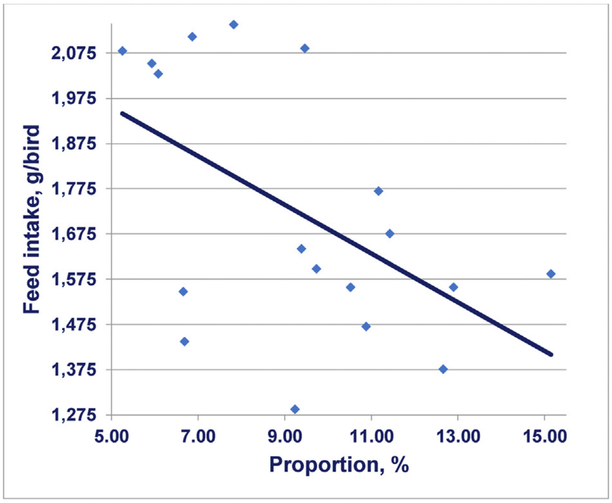

**Fig. 2.** Linear relationship ($r = -0.526$; $P = 0.025$) between proportion of uric acid-N output in excreta of dietary total-N input over the total collection period in birds offered three 165 g/kg CP diets and feed intake, where Feed intake = 2,224 − 53.8 × Proportion.

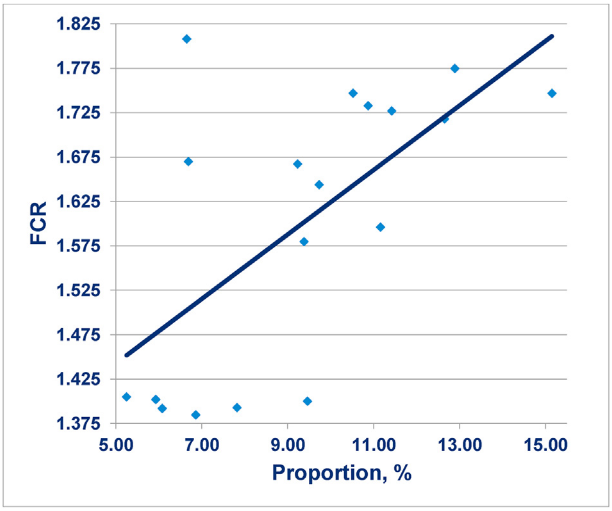

**Fig. 3.** Linear relationship ($r = 0.635$; $P = 0.005$) between proportion of uric acid-N output in excreta of dietary total-N input over the total collection period in birds offered three 165 g/kg CP diets and feed intake, where FCR = 1.261 + 0.036 × Proportion

Amino acid imbalances in reduced-CP diets create a surplus of amino acids that are catabolised (Bender, 2012) mainly by oxidative deamination in the liver, which generates $NH_3$ that requires detoxification (Stern and Mozdziak, 2019). $NH_3$ is detoxified by a condensation reaction catalysed by glutamine synthetase (Watford and Wu, 2005) in which ammonia and glutamic acid are converted into glutamine as depicted in the Minet et al. (1997) equation:

$$\mathrm{NH}_{4+} + \mathrm{Glu} + \mathrm{ATP} + \mathrm{Mg}^{2+} \Rightarrow \mathrm{Gln} + \mathrm{ADP} + \mathrm{P}i, \tag{1}$$

where ATP is adenosine triphosphate, ADP is adenosine diphosphate, and Pi is inorganic phosphorus.

Glutamine then enters the Krebs uric acid cycle whereby ammonia-N arising from deamination is ultimately excreted as uric acid-N. There is an obligatory glycine input into the Krebs uric acid cycle where one mole of glycine is required for every mole of uric acid excreted (Salway, 2018). Thus, elevated deamination of amino acids coupled with inadequate $NH_3$ detoxification would result in higher circulating $NH_3$ concentrations, or hyperammonaemia, which compromises growth performance.

The conversion of 4 molecules of ammonia into uric acid in the avian liver demands 2 molecules of glutamine, 1 molecule of glycine, 1 molecule of aspartate and 2 molecules of formyltetrahydrofolates (Mapes and Krebs, 1978). It is then relevant that there is a multiple linear regression ($r = 0.680$; $P < 0.001$) between absolute uric acid concentrations in excreta ($y$) with free plasma concentrations of glutamine ($x$) and glycine ($z$) as illustrated in the following equation:

$$y = 111.2 - 0.211 \times x - 0.613 \times z. \tag{2}$$

Thus, increasing uric acid excretion was associated with decreasing glutamine and glycine plasma concentrations in the present study, which is consistent with the Mapes and Krebs (1978) statement.

The likely genesis of amino acid imbalances in birds offered reduced-CP diets include discrepancies in apparent amino acid digestibility coefficients, diverse digestive kinetics of non-bound versus protein-bound amino acids and the possibility that non-bound amino acids are less likely to be catabolised in their transition across the gut mucosa and are more likely to enter the portal circulation (Liu et al., 2021). However, it appears that amino acid imbalances are of a greater magnitude in wheat-based, reduced-CP diets than those based on maize. This difference may be driven by the fact that wheat has the higher protein content. As a direct consequence, the 165 g/kg wheat-based diet contained 28.3% more non-bound amino acids (49.4 versus 38.5 g/kg) and less ‘intact’ soy protein as soybean meal inclusions were 57.5% less (48 versus 113 g/kg) than the corresponding maize-based diet. A further element to amino acid imbalances is that dietary CP reductions can depress monomeric amino acid transport systems but enhance the activity of PepT-1 and intestinal uptakes of di- and tri-peptides or oligopeptides, which has been reported in pigs (Qiu et al., 2016). In the present study, maize-based diets would have contained more soy oligopeptides than wheat-based diets. These differences could have amplified amino acid imbalances in the wheat-based diets, which may have been compounded by the more rapid digestion rate of wheat starch. Under *in vitro* conditions wheat starch is more rapidly digested than maize starch by a two-fold factor (Giuberti et al., 2012).

Glycine and serine are interchangeable (Sugahara and Kandatsu, 1976) and for this reason they are often expressed collectively as glycine equivalents where one glycine equivalent (g/kg) equals glycine plus serine multiplied by 0.7143 to allow for the difference in molecular weights. The importance of glycine, and by extension glycine equivalents, in fortification of reduced-CP diets was demonstrated by Dean et al. (2006). Subsequently, Siegert and Rodehutscord (2019) proposed that glycine equivalent levels should fall between 11 and 20 g/kg in diets for young broiler chicks. The broad amplitude of this recommendation was justified in part by variations in dietary concentrations of cysteine, threonine and choline impacting on glycine and serine requirements. However, every mole of uric acid excreted demands an input of one mole of glycine into the Krebs uric acid cycle (Salway, 2018). Therefore, the proportion of dietary glycine required for uric acid excretion was deduced (Table 2). Across the nine dietary treatments, the mean dietary glycine requirement was 49.2% (±45.95) with a high 93.4% coefficient of variation and a broad range from 25.0% to 80.9%. This deduction does not consider the synthesis of glycine by poultry. Glycine biosynthesis is complex and in theory both serine and threonine may serve as glycine precursors (Meléndez-Hevia and de Paz-Lugo, 2008). However, the Pearson correlations between free

**Table 5**
Pearson correlations between free plasma concentrations (μg/mL) of glycine, serine, glycine equivalents and threonine.

| Item | Glycine | Serine | Glycine equivalents | Threonine |
|---|---|---|---|---|
| Glycine | 1.000 | | | |
| Serine | $r = 0.785$<br>$P < 0.001$ | 1.000 | 1.000 | |
| Glycine equivalents | $r = 0.954$<br>$P < 0.001$ | $r = 0.935$<br>$P < 0.001$ | 1.000 | |
| Threonine | $r = 0.674$<br>$P < 0.001$ | $r = 0.574$<br>$P < 0.001$ | $r = 0.664$<br>$P < 0.001$ | 1.000 |

plasma concentrations of glycine, serine, glycine equivalents and threonine shown in Table 5 do not indicate that serine and threonine are serving as glycine precursors, as the plasma concentrations all increase in unison. It is then relevant that D'Mello, (1973) argued threonine does not serve as a glycine precursor in broiler chickens under practical conditions. Thus, the likelihood is that a substantial and variable proportion of dietary glycine is utilised in the Krebs uric acid cycle to drive uric acid synthesis and excretion and justifies the broad recommendation of 11 to 20 g/kg for glycine equivalents in reduced-CP diets proposed by Siegert and Rodehutscord (2019).

## 5. Conclusions

The proportion of uric acid-N to total N in excreta in birds offered the 165 g/kg CP, maize-based diets was significantly lower by 10.6 percentage units (27.4% versus 38.0%) than their direct wheat-based counterparts. While not conclusive, this outcome is consistent with the suggestion that reduced-CP, wheat-based diets amplify amino acid imbalances in comparison to maize-base diets, leading to the deamination of surplus amino acids and accumulation of ammonia. Thus, the grossly inferior growth performance of birds offered reduced-CP, wheat-based diets may be attributed in part to ammonia overload. Therefore, every endeavour should be made to meet amino acid requirements precisely in the formulation of reduced-CP diets.

## Author contributions

**David Cantor**, **Leon McQuade** and **Bernie McInerney** were all involved in developing and validating the analysis method to determine uric acid concentrations in avian excreta. The remaining authors were all involved in the original feeding studies from which uric acid excreta concentrations were retrospectively analysed. **Sonia Yun Liu**, **Shemil Macelline** and **Peter Selle** were responsible for the statistical analysis and interpretation of the recent data and were mainly responsible for completion of the manuscript.

## Conflict of interest

We declare that we have no financial and personal relationships with other people or organizations that can inappropriately influence our work, and 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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