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Background

Crude Protein (**CP**) levels of German broiler feed declined continuously from 20.8% weighted average CP of total consumed (**WACP**) in 2000 to 19.3% WACP in 2020 (Emthaus et al., 2021). During same period, final body weights of broilers increased, and feed conversion ratio decreased. Consequently, nitrogen (N) excretions per kg live weight or edible product decreased by 35% or 38%, respectively. Moreover, the authors proposed further dietary CP reduction until 2030 which would mean a reduction of N-excretions by 47% (live weight) or 47% (edible product) compared to the year 2000. Accordingly, N-utilization was 60% in 2020 and is expected to achieve 65% in 2030. Thus, broilers are very efficient N-converters which is confirmed by others (Fry et al., 2018; Poore and Nemecek, 2018; Uwizeye et al., 2020).

Uwizeye et al. (2020) reported that N-emissions by livestock account for about "one-third of global human-induced N-emissions". Although ruminants represented 75% of the total livestock N-excretions, intensive broiler chicken systems accounted for 5,366,349.179 t N/year which is equal to 5.4% of total global livestock N-excretions (Uwizeye et al., 2020). Despite the proportion of N-excretions from broilers in Germany was only about 0.6% of above-mentioned global excretions, the paper suggests that Germany and other central European countries belong to hotspots of N_2O and NH_3 emissions and also of NO_3^- output by animal production. Because of problems related to that, e.g. quality of ground and drinking water, legislation in Germany has been revised in order to monitor, control and finally reduce N-emission from livestock (Bundesministerium für Umwelt et al.; Federal Ministry of Justice and Consumer Protection, 2017a, 2017b). Accordingly, 357 g N excretion per place and year are assumed for broilers fed N-reduced feed (Federal Ministry of Justice and Consumer Protection, 2017b). However, a recent evaluation of data collected from entire Lower Saxonia, Germany, suggested 384 g/year/place with N-reduced feed instead (Wilkens, 2022).

The study by the German Association Animal Food documents steady successes in reduction of dietary CP levels and, thus, reduction of N-excretions (Emthaus et al., 2021). While an increasingly growing understanding of nutrient requirements as well as improved analytical methods to evaluate feed components helped a lot to optimize compound feed close to the animal's requirements, particularly increasing number and available quantity of free amino acids as feed supplements for compound feed production allowed a reduction of dietary CP while meeting the amino acid requirements at the same time. The use of free amino acids improves feed conversion ratio and, thus, the demand of feed while it also reduces the inclusion of soybean meal (Selle et al., 2020; Sturm et al., 2021; Sturm et al., 2022). DL-Methionine (**DL-Met**) was first produced in the late 1950s, L-Lysine (**L-Lys**) fermentation process started in the 1960s, followed by L-Threonine (**L-Thr**) and L-Tryptophan (**L-Trp**) and more recently L-Valine (**L-Val**) , L-Arginine (**L-Arg**) and L-Isoleucine (**L-Ile**) (Sturm et al., 2021; Sturm et al., 2022). An increasing number of free amino acids increases flexibility in feed formulation and precise adjustment of the dietary amino acid profile. In this context, it might be worth mentioning that despite the fact that they are registered as feed additives in the European Union, particular the use of L-Arg and L-Ile in broiler feed is not yet well established while at least sometimes, L-Val can be detected (Evonik Operations GmbH, 2016).

While scientific literature reports many broiler feeding studies about dietary CP reduction (Lemme and Rodrigues, 2020), applied CP levels in commercial German broiler diets are seldomly below those reported by Emthaus et al. (2021). There are probably two main concerns. On the one hand, price scenario of feed ingredients and amino acid supplements often result in increasing feed prices at lower (forced) dietary CP levels especially if protein sources are relatively cheap. Price scenarios for protein sources in 2022 instead would improve cost effectiveness of protein reduction. On the other hand, feed and livestock industry fears performance losses, particularly impaired feed conversion ratio and meat deposition with low CP diets as suggested by Pesti (2009). Indeed, translating scientific findings into commercial application bears such risk as conditions are often less controlled compared to scientific conditions. The more the dietary CP is reduced, the more essential amino acids become limiting at the same time. This means if only one amino acid is lower than intended, performance of birds may suffer.

However, recent research could show significant progress in protein reduction. The findings by Chrystal et al. (Chrystal et al., 2020; Chrystal et al., 2021) seem to indicate a milestone in this context. They reduced dietary CP from 20.8% (positive control) to 16.5% (negative control) in corn-based diets for 7-35 day old broilers and added L-Thr and/or Glycine equivalents (**Gly-equivalents**) to the negative control (Chrystal et al., 2020). Only the combination of both amino acid supplements brought performance back to the level of the positive control while adding only L-Thr (as a theoretical precursor of Gly) or Gly-equivalents were not successful. Obviously, CP reduction per se affects the threonine metabolism and Liu et al. (2021) speculated that an increased metabolic availability of acetyl-CoA due to higher starch and glucose load in low CP diets down-regulates the activity of threonine-3-dehydrogenase (TDH). This would result in reduced threonine (**Thr**) metabolism and increased plasma Thr concentrations (Liu et al., 2021) but also suggest, that Thr as precursor for de-novo synthesis of Gly would not be effective. However, increasing dietary Thr supply under low CP conditions would counterbalance this effect on TDH while the addition of Gly-equivalents would be required to meet the requirement of these conditionally essential amino acids especially in young broilers (Hilliar et al., 2019). Question remains why CP reduction to 16.5% was not as successful in wheat based diets (Chrystal et al., 2021; Greenhalgh et al., 2020). While the researchers compared the diets and found that low protein wheat-based diets contain higher amounts of free amino acids and lower inclusion of soybean meal which might have had implications on digestion dynamics and synchronicity of absorption and utilization of amino acids and glucose along the gastrointestinal tract (Chrystal et al., 2021; Liu et al., 2021), different grains would also have impact on microbiota which may interact with the host as well (Wang et al., 2021).

In Germany, broiler feed often uses high amounts of wheat. Protein reduction trials with broilers were conducted at German research facilities using pens with 250 (Lemme, Hiller et al., 2019) or 150 (Lemme, Wild et al., 2019) broilers per pen and, thus, simulating fairly well commercial conditions. Referring to German recommendations (Hiller, 2014) for standard (20.4% WACP) or N-reduced feed (19.5% WACP), Lemme, Hiller et al. (2019) further reduced dietary CP in grower 1, grower 2 and finisher diets resulting in treatments with 18.7% WACP and 17.9%

WACP. Performance of mixed-sex broilers of all treatments was well ahead breeder recommendations at day 40, but, however, the lowest protein treatment had significantly lower body weight and breast meat deposition (females) compared to the other treatments. It appeared that the growth curve of the lowest WACP treatment started to deviate from the others after introduction of finisher feed with 17 % CP. Extra L-Thr was put into the diet for balancing out a Gly-equivalent gap but with today's knowledge (see above) we would not recommend this strategy anymore. The second (German recommendation) and third N-reduced treatment were successfully repeated in another trial (Lemme, Wild et al., 2019). Both trials have in common that litter weight decreased and quality improved with N-reduction resulting in improved footpad health (Lemme, Hiller et al., 2019). Also, N-utilization significantly improved, and N-excretions were reduced at the same time (Lemme, Hiller et al., 2019; Lemme, Wild et al., 2019). The rule of thumb that reduction of dietary CP by 1%-point would reduce N-excretions by 10-12% was confirmed. The treatment "N-reduced according to German recommendations" is close to commercial practice in recent years (Emthaus et al., 2021) and, thus, these trials already indicated a certain potential for N-reduction in commercial broiler production.

Aim of the project

A project was initiated to apply dietary CP reduction under commercial conditions. The main objective was to demonstrate and validate that dietary CP can be reduced without compromising broiler performance in a production system which is already rather efficient. In addition, we wanted to demonstrate the potential of dietary CP reduction on reducing N-excretions especially in the context of German revised regulations and monitoring attempts. Finally, as previous research suggested, few further aspects such as impact of dietary CP reduction on litter quality and quantity, footpad health, change of ingredient inclusion levels and related impact on sustainability impact factors were evaluated.

Experimental set-up

A farm with 10 barns (1800 m^2; 23 birds/m^2; four feeding lines; 6 nipple drinker lines) with about 42,000 broilers each was available for three large scale trials. Day old chicks were placed at the same day in all 10 houses of the farm while broilers were harvested in three crops. A first thinning took place at day 29 (~ 1500 g/bird), a second thinning at day 34 (~ 2000 g/bird) and the main crop took place at days 41-42 (~2700 g/bird). While in crop 1 and 2 about 16 % each of overall produced liveweight was harvested, 67% of overall liveweight production was harvested at the main crop (Fenske et al., 2019). At day one, birds received 24 hours of light which was reduced to 22 hours on days two and three and thereafter it was reduced to 18 hours. Basically, broilers received a standard vaccination program against Infectious Bronchitis and Newcastle disease. Corn silage (0.56kg/m^2) was used as bedding and no additional bedding was put into the barns during course of the experiment.

In a four-phase feeding program (starter: days 1-10, grower I: days 11-20, grower II: days 21-25, finisher: days 26-harvest) feed and water was provided *ad libitum*. Feed was produced in a commercial feed mill. Because the first thinning at day 29, finisher diets free of coccidiostats were introduced already at day 26. In all trials, wheat-corn-soybean meal-based diets were formulated according to commercial practice, using ingredients available at that time and adjusting diet composition to the respective ingredient price scenario. Accordingly, treatment 1 (TRT 1) represented always the commercial standard feeds while in TRT 2 CP levels were reduced in grower I, grower II, and finisher feeds. Crude protein was not reduced in starter feeds to minimize the risk of marginal supply of nutrients during the important first days of life. For CP reduction only registered and commercially available amino acids were used. While the feed producer used DL-Met, L-Lys, L-Thr and L-Val on regular basis for standard feed, CP reduction could be realized by using L-Arg and L-Ile. For CP reduction Evonik AMINOChick® 3.0 recommendations (Lemme, 2021) were considered which means also Gly-equivalent were monitored. In grower 1, grower 2 and finisher diets 5, 10, and 12% unground wheat were included. However, this was not a dilution of pelleted compound feed but whole wheat addition was considered in feed formulation. From each produced batch of feed, samples were taken for analysis adding up to a total of about 100 samples per trial. Analysis of amino acids indicated that expected amino acid levels were exactly met in each trial. Moreover, there was no variation between samples within treatment and phase indicating that commercial feed production can be very precise, provided the nutritional value of the ingredients is well understood. Analysed CP (N) levels were used for N-balancing calculations. All diets contained phytase and xylanase. Feed consumption was recorded after each feeding phase according to feed deliveries into the silos and remainders after final harvest was deducted as well as it was made sure that silos were empty at filling with starter feed. Body weights and number of birds were recorded over the entire production cycle for each barn as reported from the slaughterhouse. Average body weights, average feed intake, feed conversion ratio (body weights of losses considered) and mortality were calculated accordingly. The slaughterhouse also reported footpad lesion scoring determined by camera scanning for all birds following a four-grade scoring system: 0 = no lesions (1-5% of pad area), 1 = 6 – 20 % of pad area with lesions, 2a = 21 – 50 % of pad area with lesions, and 2b > 50 % of pad area with lesions. Other information regarding carcass composition and quality could not be obtained.

In trial 1, CP was reduced moderately in experimental diets. Accordingly, standard starter, grower 1, grower 2 and finisher diets contained 21.6, 20,6, 20.1, and 19.3% CP while experimental grower 1, grower 2 and finisher diets contained 20.1, 19.9, and 18.8% CP (all analysed according to Dumas) resulting in a WACP reduction of 0.3%-points. Experimental diets were mainly based on wheat, corn, soybean meal and some rapeseed products. In this trial also up to 9% field peas were used as peas were available from the market. For CP reduction inclusions of wheat and soybean meal were varied resulting in a reduction of soybean meal in overall consumed feed per bird by 1.9%-points (-9% on relative scale).

In trial 2, a finisher 2 diet was introduced in the experimental treatment. Analysed CP in standard starter, grower 1, grower 2 and finisher diets was 22.8, 21.0, 20.2, and 19.1% whereas experimental grower 1, grower 2, finisher 1 and finisher 2 diets contained 20.3, 19.5, 18.4 and 17.9% CP. The difference in WACP was 0.7%-points and average soybean meal reduction was 2.4%-points (-10% relative). In this trial no peas were used. Finisher 2 feed was introduced at day 38 and was, thus, consumed only from broilers harvested with main crop.

In trial 3, the low protein treatment also included a finisher 2 diet. However, in this case blending units, which were available in the barns, were used and proportions of finisher 1 and finisher 2 feeds were gradually changed on a daily basis. Analysed CP in standard starter, grower 1, grower 2 and finisher diets was 22.3, 21.2, 20.9, and 19.1% whereas experimental grower 1, grower 2, finisher 1 and finisher 2 diets contained 20.6, 19.4, 18.7 and 17.5% CP. CP. The difference in WACP was 1.1%-points and average soybean meal reduction was 3.8 %-points (-17% relative). In addition, the amino acid concept changed insofar as in low protein diets, SID Thr:SID Lys was increased by 5%-points compared to AMINOChick® 3.0 recommendations.

A fourth trial was conducted but not on the farm but in a research facility which was also used earlier in other projects (Lemme, Hiller et al., 2019). The reason behind was that in this case also glycine was added for balancing the amino acid profile at even stronger CP reduction. In the EU, Gly is not registered as feed additive but only as flavor and the applied supplementation exceeded allowed doses as flavor (https://ec.europa.eu/ food/safety/animal-feed/feed-additives/euregister_en). Five pens with mixed-sex Ross 308 broilers per treatment were available. Analysed CP in standard starter (1-10d), grower 1 (11-17d), grower 2 (18-28d) and finisher diets (29-34d) was 21.9, 20.1, 20.1, and 18.7% whereas experimental grower 1, grower 2, and finisher 1 diets contained 19.5, 18.4, and 17.2% CP. The difference in WACP was 1.5%-points and average soybean meal reduction was 7.5 %-points (-34% relative). In this trial, one batch of feed per phase and treatment was produced by a commercial compound feed manufacturer.

Biological performance was not affected by dietary protein reduction

In all trials dietary CP reduction did not affect growth performance nor feed conversion ratio. This is true also for each single crop in the large-scale trials (1-3) as well as for all recordings at day 10, 29, and 34 in the pen trial. Accordingly, average final body weights (**FBW**) were 2344 g, 2320 g, and 2278 g/bird and respective feed conversion ratios (**FCR**) were 1.52 kg/kg, 1.54 kg/kg and 1.54 kg/kg in trials 1-3 with standard feed. With protein reduction, FBW were 2328 g, 2330 g, and 2372g g/bird while FCR were 1.50 kg/kg, 1.55 kg/kg, and 1.52 kg/kg. Average slaughter age across all 3 crops was 36.7, 36.2, and 36.8 days for both treatments, respectively. In trial 4 birds were slaughtered at 34 days of age and achieved a final body weight of 2355g or 2349g with standard or CP reduced feed with respective FCR of 1.48 kg/kg or 1.47 kg/kg. It can, therefore, be concluded that at even rather intensive commercial conditions dietary CP reduction is possible up to 1.0 to 1.5 %-points WACP without affecting performance. Moreover, while lowest CP reduction in the above mentioned earlier trial (Lemme, Hiller et al., 2019) failed to maintain the performance, results of trial 4 conducted in the same facility showed successfully that performance could be maintained. Indeed, the earlier trial lasted 41 days, while the current trial ended at day 34, but dietary CP levels of finisher feeds were very similar. One reason for this might have been, that in trial 3 and 4 the concept of enhanced SID Thr: SID Lys was introduced. Obviously, also use of wheat as one major grain did not prevent successful dietary CP reduction and it is concluded that this is probably a challenge in extremely low CP diets as used by Chrystal et al. (2021).

Nitrogen emissions were reduced by dietary protein reduction

As described above, soybean meal inclusion could be reduced by up to 34%. Selle et al. (2020) reported a reduction of soybean meal of 66% (33.4% vs 11.3%) in corn-based grower diets with 16.5% CP. Although few more (non-registered) amino acids were used for balancing, this suggests still a big potential for commercial feed production today. In diets used in our trials, soybean meal inclusion in standard feed was not as high as 33.4% but achieved average levels of 20 to 24% in starter, grower and finisher diets and, therefore, reductions by 17.0% to 18.3% (trial 3) or 34.0% to 14.6% (trial 4) appear remarkable and are in line with earlier findings (Lemme, Hiller et al., 2019; Lemme, Wild et al., 2019).

Ingredient compositions were assessed for their global warming potential by multiplying ingredient inclusion levels with sustainability impact figures from GFLI database (https://globalfeedlca.org/gfli-database/). Accordingly, soybean meal reduction would have a major impact - at least if calculations consider “land use change” (**LUC**) because soybean meal used in Germany is mainly sourced from South America. Global warming potential (**GWP**) could be reduced by 4, 5, 8, and 15% in trials 1 – 4 and indeed soybean meal reduction contributed most to this effect. Basically, only soybean meal contributed between 58 and 62% to overall GWP in standard feeds of the trial while this proportion reduced to 48 and 59% with dietary CP reduction. However, when not considering LUC, GWP would not show any beneficial effects of CP reduction anymore. In 2015, Germany and six further European countries signed a declaration to promote elimination of deforestation and to support the goal of zero net deforestation of rainforest (Hansen et al., 2015). In 2020, 57% (46% in 2019) of the domestic soybean meal consumption of Germany was reported to be in line with the soy sourcing guideline of the European Feed Manufacturers' Federation (FEFAC) (2021) for “conversion-free soy” while 37% (25% in 2019) of domestic use were reported to be certified (IDH - sustainable trade initiative, 2022). Thus, the effect of LUC in life cycle analyses is increasingly reduced in this context.

Calculation of eutrophication and acidification potentials of the experimental diets revealed that CP reduction had no effect. However, it should be noted that dietary CP reduction would especially reduce N-excretions and, thus, the major impact occurs in the animal house which could be determined by lifecycle analysis taking live weight or meat yield as functional units. In a recent exercise in which CP reduction in broiler feeds of different regions was assessed, eutrophication potential referring to liveweight production was reduced by 10% particularly because of effects on manure storage and application – same applies to acidification (-13%) (Stubbusch and Binder, 2021).

With standard feeds, N-utilization was 61, 60, 61, and 64% in trials 1, 2, 3, and 4, respectively. Interestingly, Uwizeye et al. (2020) suggested a N-utilization of 61% for Germany which was the highest value for all reported 217 countries. For Europe, respective utilization was 59% and for overall global production 55% when considering intensive broiler production (Uwizeye et al., 2020). N-utilization increased to 63*, 62, 65*, and 69*% (*: $p < 0.05$) in trials 1-4 with dietary CP reduction. Accordingly, N-excretions were significantly ($p < 0.05$) reduced by 9, 8, 11, and 21%. Nitrogen excretions ranged between 315 and 329 g N/place/year (7.5 cycles per year; 42 days + 7 empty days per cycle) when feeding standard diets in trials 1 to 3 whereas dietary CP reduction reduced N-excretions further to 301, 302, and 281 g N/place/year. Compared to 384 g N/place/year reported for N-reduced broiler nutrition in Lower Saxonia, Germany, for 2021, the excretions of the current reduced CP treatments were 22-27% lower (Wilkens, 2022). Trial 4 was shorter and would suggest 361 g N/place/year with standard feed (9 cycles; 34 days + 7 empty days per cycle). CP reduction in feed then reduced this number to 285 g N/place/year. The regulation on nutrient balancing (Federal Ministry of Justice and Consumer Protection, 2017a) allows entering produced broiler liveweight as well as consumed feed with declared or analysed CP levels which then will allow to calculate the N-output. Therefore, the dietary CP reduction would directly positively affect the farm gate balance requested by the authorities. A more recent regulation deals with emissions from animal production such as ammonia (Bundesministerium für Umwelt et al., 2021) and dietary CP reduction can reduce such emissions as well as previously suggested (Belloir et al., 2017; Hernandez et al., 2013).

Feet of all birds delivered to the slaughterhouse were scored for footpad lesions. All 3 large scale trials indicated improved footpad health with CP reduction. While there was no issue with standard feed per se, proportion of Grade 0 score increased in trial 1 ($p < 0.10$), trial 2 ($p < 0.05$) and trial 3 (numerically) which is in line with earlier findings (Lemme, Hiller et al., 2019). Reduced N-excretion, which would require less water by the metabolism, as well as reduced soybean meal inclusion, which reduces the dietary potassium load, result in higher dry matter content of the litter and therefore prevents aggressive fluids (Lemme, Hiller et al., 2019). Indeed, reduced water consumption was recorded at least in trial 1. However, while not significant, CP reduction reduced litter weight by 12% in trial 1, 6% in trial 3 and 31% in trial 4 (no results from trial 2) being in line with earlier findings (Lemme, Hiller et al., 2019; Lemme, Wild et al., 2019).

Conclusions

From these trials conducted in a commercial environment including a commercial feed manufacturer, it can be concluded that – compared to current German standard – dietary CP can be reduced by 1.0-1.5%-points on average without compromising broiler performance. Moreover, although already on top of an international ranking (Uwizeye et al., 2020), N-utilization can be further increased and N-excretions can be considerably reduced. Thus, CP reduction is an efficient tool for minimizing N-emissions. At the same time, dietary inclusion of protein-rich feed ingredients will be reduced. Ecological footprint of the feed is not improved by dietary CP reduction particularly when land use change is excluded (or if e.g. certified soybean meal is used) whereas the ecological footprint of liveweight production (not determined in this project) would likely improve due to reduced N-excretions. Moreover, CP reduction showed also clear benefits with respect to footpad health as well as to litter quality and quantity. With regard to the outlook by Emthaus et al. (2021) it can be concluded that the suggested achievements in N-excretion reduction for 2025 or 2030 are already realizable today.

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