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Article

Scaling Up the Effects of Low Nitrogen in Commercial Broiler Farms

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Abstract: In a protein reduction feeding trial (Study 1) on a commercial broiler farm in northern Germany, it was attempted to be shown that research results from station tests on protein reduction can be transferred to agricultural practice. In a second study, the limits of the N reduction were tested in a research facility. In Study 1, commercial standard feeds were fed to the control group (variant 1:210,000 animals; $n = 5$ barns). In the test group (variant 2:210,000 animals; $n = 5$ barns), the weighted mean crude protein (CP) content was moderately reduced by 0.3%. The nitrogen reduction in the feed did not affect performance (feed intake (FA), daily gain (DG), feed conversion (FCR)), but nitrogen conversion rate increased from approx. 61% to approx. 63%. The solid litter weight was reduced by 12% and nitrogen excretion by 9% ($p < 0.05$). Significantly healthier footpads were due to lower water intake (−4%; $p < 0.05$) and a numerically drier bedding. In Study 2, responses of treatments (1250 broiler per variant; $n = 5$) showed that sharper N-lowering (−1.5% CP; weighted average) did not impair performance either, but N-conversion improved and N-excretions decreased significantly. Converted to a protein reduction of one percentage point, the N excretions were able to be reduced by 22% in Study 1 and 18% in Study 2. Feeding trials in the commercial sector, such as the present Study 1, should convince feed mills and farmers to allow the latest scientific results to be used directly and comprehensively in commercial ration design.

Keywords: low protein; nitrogen; amino acid; commercial farms; broilers; ammonia

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1. Introduction

In order to achieve the climate targets that have been set, it is necessary to reduce emissions not only in industries, but also in animal husbandry. In particular, the ammonia emissions and the locally high levels of nitrogen (N) in soils in animal-intensive regions can be influenced primarily by feeding. This was recommended by Santonja et al. [1] at the EU level. Politics constantly set new standards for the animal feed industry and animal husbandry companies in order to achieve these ambitious goals as quickly as possible. At the national level, regulations have been revised and newly issued in Germany in order to influence nitrogen emissions from agriculture [2–4]. At the European level, the EU NEC Directive 2016/2284 (National Emission Ceilings Directive) is relevant, which stipulates ammonia reductions of 29% for Germany by 2030 compared to the reference year 2005 [5].

Analyses by DVT [6] showed that both the national and international targets for (N) savings can be achieved in the short to medium term. The broiler already utilizes N particularly efficiently compared to other animal species [7]. In addition, DVT [6] calculated a greater potential for N savings by 2030 for the German broiler feed industry (42% compared to the reference year 2005) than for pigs (32% compared to the reference year 2005). However, broilers do not need N per se, but amino acids (AA), which account for about 90% of the nitrogen [8]. Ideally, the AA in the feed are present in a profile that is optimal for the animal species and age. With dietary N-reduction, more and more AA approach this ideal protein profile and at the same time become performance-determining, while relative excesses are reduced [9]. If the needs of one or more AA then change, for example, due to an infection, the resulting marginal supply situation can potentially lead to a drop in performance. Therefore, when the protein content in the ration is close to the broiler's physiological optimum, all other production factors must be optimized to ensure the exact delivery of the nutrients. Important framework conditions in feed production include, for example, the mixing accuracy in the feed factory, the correct quality assessment of the feed ingredients and possible decomposition during transport, storage in the silo, or in the feed screw in the barn.

Many scientific studies have shown that a significant reduction in the protein content in chicken feed is possible. However, small groups, such as 42 [10] to 288 animals [8] per variant, have often been examined for this purpose. It cannot be guaranteed that the results from tests in a scientific institution are transferable to a commercial operation with practical production conditions—one reason for the cautious adoption of scientific knowledge in commercial applications.

Two studies within a larger project were conducted to determine the effects of commercial complete feed with and without N reduction on performance, health and nutrient utilization. Both studies were based on optimized ration design with a focus on balancing digestible AA. The aim of the first study was to recreate the verified effects of moderate N reduction achieved in station tests in practice. A stronger N reduction in Study 2 should further test the limits of N reduction at a constant fattening and slaughter performance to generate scientific knowledge before new legislation requires a reaction.

2. Materials and Methods

2.1. Animals and Housing

Study 1: On a commercial farm in Northwest Germany, 420,000 day-old mixed-sex Ross 308 chicks were randomly assigned to 10 barns with 42,000 animals each. Five barns received a commercial standard control feed, and the other five barns, a protein-reduced test feed. The goal was to equalize the average parent flock, and chicks from different parent flocks were divided between the two feed variants. Correspondingly, the weighted mean of the chicks came from parent flocks of production weeks 9.73 (standard) and 9.23 (low protein; Table 1). Random sampling of 100 chicks per house resulted in similar average barn weights of 41.1 ± 3.3 g/chick or 40.9 ± 3.3 g/chick for the standard and experimental variants, respectively. The temperature program was in line with the breeder's recommendations [11]. The broilers received 24 h of light on day 1, 22 h on days 2 and 3, and 18 h of light from day 3 to day 42. The light intensity was approx. 80 lux on day 1 and was reduced by 5 lux every 3–4 days, so that from day 14 onwards the barns were illuminated with 60 lux. Broilers received a standard vaccination program against infectious bronchitis (days 1 and 13), Newcastle disease (day 7) and gumboro (day 13). The barns had a size of 1800 m^2 (23 animals/m^2) and were each equipped with four feeder lines and six nipple drinker lines for the ad libitum supply of feed (82 animals per trough) and water (14.5 animals per drinker). Dried maize silage was used as bedding (0.56 kg/m^2; no additional bedding). Cropping of broilers was organized in such a way that already 24% of broilers equivalent to about 16% of the overall produced live weight were harvested at day 29. A second thinning took place at day 34 where a further 20% of broilers (17% of final overall live weight) was cropped. The main harvest took place at days 41 and 42 (56% of the broilers and 67% of the live weight). With this procedure, a maximal density of 35 kg live weight/m^2 were not exceeded at any point in time.

Study 2 was conducted at Haus Düsse, North Rhine-Westphalia Chamber of Agriculture, where 5 pens with 250 day-old mixed-sex Ross 308 broilers each (as hatched; n = 1250) were examined. The average chick weights were 41.0 g (standard) and 40.8 g (experimental). The temperature program, as well as the light management and the light intensity corresponded to the good professional standard and the recommendations of the breeder [11]. Vaccinations against infectious bronchitis (days 1 and 13), Newcastle disease (day 7) and gumboro (day 13) were given. Each pen was equipped with a drinking line with 20 drinking nipples and 4 automatic feeders. In the first few days, the 4 automatic feeders were supplemented by 3 flat cardboard trays with starter feed. Lignocellulose was used as bedding and added to all boxes as the bedding became damp.

Table 1. Age of parent stock flocks and average chicken weight at placement (Study 1, n = 100 chicks per house).

Barn	Group	Parent Stock Flock	Week of Production	Number of Chicks Placed	Average Weight, g/Chick
1	standard	A	4	42,000	39.15
2	low protein	A	4	8560	40.83
2	low protein	B	7	33,440	
3	standard	B	7	42,000	40.92
4	low protein	B	7	19,170	
4	low protein	C	9	22,830	41.49
5	low protein	C	9	42,000	
6	standard	C	9	12,431	40.36
6	standard	D	12	29,569	
7	standard	D	12	31,413	41.53
7	standard	E	11	10,587	
8	standard	E	11	32,313	41.46
8	low protein	E	11	9687	41.73
9	low protein	E	11	32,888	
9	low protein	F	14	9112	40.09
10	standard	F	14	24,973	
10	standard	G	16	17,027	
Weighted average production week		standard	9.73		41.10
		low protein	9.23		40.90

2.2. Diet Preparation, Calculations and Chemical Analyses

Study 1: The broilers in Study 1 were fed a 4-phase feeding program: starter (day 1–10), grower diet I (day 11–20), grower diet II (day 21–25) and finisher diet (day 26–42). Because coccidiostats had to be discontinued at least 3 days before slaughter to avoid residues in the meat, a finisher feed free of coccidiostats was used from day 26 onwards. The 4 feeds of the control group complied with the commercial standard of the feed supplier at the time of the experiment. While the CP content in the starter feed was identical for both treatments, it was reduced in the test group from grower I feed onwards. When optimizing the test diets, care was taken to ensure that only components that are permitted under feed law in Germany were used. In addition to the AAs DL-methionine, L-lysine, L-threonine and L-valine, which were used as a standard by the feed manufacturer, L-isoleucine and L-arginine were also used in the test group. The digestible amino acid profiles were based on the recommendations of AMINOChick 2.0 [12]. A moderate protein reduction was chosen to minimize the risk of commercial losses at the location of Study 1.

The pelleted compound feeds for Study 1 (1400 t compound feed) based on wheat–corn–soybean meal were produced in a commercial feed mill. Peas were available at the time of the trials, so they were mixed in at a rate of 9% (Table 2). For all barns, unground wheat was mixed in at the factory (in the 4 feed phases with 0%, 5%, 10% and 12% whole wheat content) and was included in the nutrient optimization so that no dilution took place. The addition of whole wheat increases digestive activity, increasing the size of the gizzard and also the absorption surface area of the small intestine. Since the digestive tract is an important part of the immune system, the addition of whole wheat is said to have a health-promoting effect [13,14]. Furthermore, the addition of wholegrain wheat has an energy-saving effect in feed production because it is added after pelleting.

Table 2. Ingredients and calculated (N-analyzed) nutrient composition of the diets in Study 1.

	Starter	**Grower I**		**Grower II**		**Finishers**	
		Control diet (standard)	Experimental diet (N reduced)	Control diet (standard)	Experimental diet (N reduced)	Control diet (standard)	Experimental diet (N reduced)
Ingredients (%)							
Wheat-unground		5.0	5.0	10.0	10.0	12.0	12.0
Wheat	25.7	24.7	27.4	24.6	26.4	22.4	24.3
Corn	38.0	28.5	28.5	25.2	25.2	24.7	24.7
Bakery meal		2.8	2.8			2.6	2.6
Soybean meal	23.3	23.8	21.1	20.7	18.8	18.3	16.4
Soybean concentrate	3.0						
Rapeseed full fat	3.0	3.3	3.3	1.8	1.8	1.8	1.8
Rapeseed expeller				1.5	1.5	1.7	1.7
Field peas		4.8	4.8	9.0	9.0	8.8	8.8
Vegetable oil	2.1	3.3	3.0	4.0	3.8	5.1	4.8
Lysine sulfate 70	0.63	0.33	0.46	0.29	0.38	0.28	0.37
DL-Methionine	0.40	0.33	0.35	0.30	0.31	0.28	0.29
L-Threonine	0.16	0.11	0.15	0.10	0.13	0.10	0.13
L-Valine	0.08	0.05	0.10	0.04	0.08	0.04	0.07
L-Arginine			0.08		0.06		0.06
L-Isoleucine			0.05		0.04		0.04
Minerals and Premix [a]	to 100	to 100	to 100	to 100	to 100	to 100	to 100
Energy (MJ AMEn/kg) and Nutrients (%)							
AMEn, MJ/kg	12.6	12.9	12.9	13.0	13.0	13.3	13.3
Ether extract	5.9	7.1	6.9	7.3	7.1	8.4	8.1
Crude fiber	2.8	2.8	2.8	3.0	2.9	2.9	2.8
Ash	5.3	4.7	4.6	4.6	4.5	4.2	4.1
Starch	40.7	40.5	41.9	41.7	42.7	42.3	43.4
Crude protein—expected [b]	21.0	20.2	19.5	19.6	19.1	18.6	18.1
Crude protein—analyzed [c]	21.6	20.6	20.1	20.1	19.9	19.3	18.8
Lysine [d]	1.40 (1.39)	1.20 (1.20)	1.20 (1.20)	1.17 (1.16)	1.15 (1.15)	1.09 (1.08)	1.08 (1.08)
Methionine + Cysteine [d]	0.99 (1.02)	0.91 (0.93)	0.89 (0.92)	0.86 (0.89)	0.88 (0.88)	0.83 (0.84)	0.82 (0.84)
Threonine [d]	0.89 (0.89)	0.81 (0.82)	0.81 (0.81)	0.78 (0.78)	0.78 (0.78)	0.74 (0.76)	0.74 (0.74)
Arginine [d]	1.34 (1.33)	1.29 (1.30)	1.30 (1.30)	1.28 (1.27)	1.29 (1.27)	1.19 (1.19)	1.20 (1.19)
Valine [d]	1.00 (1.02)	0.95 (0.96)	0.96 (0.96)	0.93 (0.93)	0.93 (0.92)	0.87 (0.88)	0.87 (0.87)
Isoleucine [d]	0.86 (0.85)	0.82 (0.82)	0.82 (0.82)	0.80 (0.79)	0.81 (0.79)	0.75 (0.75)	0.75 (0.74)
Glycine $_{\text{equivalents}}$ [d,e]	1.56 (1.53)	1.49 (1.50)	1.41 (1.41)	1.46 (1.46)	1.43 (1.40)	1.38 (1.38)	1.32 (1.32)
SID Lysine	1.27	1.08	1.09	1.04	1.04	0.97	0.97
SID Methionine + Cysteine	0.94	0.85	0.85	0.80	0.80	0.76	0.76
SID Threonine	0.78	0.70	0.70	0.67	0.67	0.64	0.64
SID Arginine	1.20	1.18	1.18	1.15	1.15	1.08	1.08
SID Valine	0.91	0.85	0.85	0.81	0.82	0.77	0.77
SID Isoleucine	0.76	0.73	0.73	0.70	0.70	0.66	0.66
SID Glycine $_{\text{equivalents}}$	1.32	1.28	1.21	1.25	1.19	1.18	1.13

[a] Contains phytase and xylanase, starter and grower diets contained coccidiostats; [b] according to Kjehldahl; [c] analyzed according to Dumas; [d] analyzed values, in brackets: expected; analyses based on starter feed: *n* = 7; Growers I: *n* = 14/16; Growers II: *n* = 12/8; Finisher feed: *n* = 23/17 for standard feed/experimental feed, respectively [e] Gly $_{\text{equivalents}}$: Gly + 0.814 × Ser.

Since AAs, which are currently not registered were required for a stronger reduction in protein, Study 2 was carried out in a test facility. This allowed the limits of the CP reduction to be exhausted. Thus, an average protein reduction of 1.5% CP (weighted average) compared to the commercial standard was achieved in the feed. The procedure for feed formulation and compound feed production in Study 2 was identical to Study 1. The only exceptions were threonine (Thr) and glycine (Gly). There was no glycine supplementation in Study 1 because the glycine equivalence level at the planned protein levels in the experimental variant of 21.0/19.5/19.1/18.1 did not have a limiting effect and the use of glycine in commercial practice is not permitted [15]. Due to an average

CP reduction of 1.5%, glycine supplementation was necessary and an average of 1.5 kg glycine/t was used to achieve a SID Gly:SID Lys ratio of approx. 1.2:1 (SID = standardized ileal digestibility). In addition, the SID Thr: SID Lys ratio in the test feed (Grower 1, Grower II, Finisher) was increased by 5 percentage points compared to the standard feed because Chrystal et al. [16] demonstrated that 0.75:1 in Thr:Lys ratio and 1.15:1 in Glyequi:Lys ratio allowed for maintaining performance when reducing CP from 20.8% to 16.5% in grower diets.

In Study 2, the whole wheat was added before pelleting because feed manufacturer logistics. In order to maintain the positive effect of whole wheat on intestinal health despite pelleting, which leads to partial destruction of the grains, the proportion of whole wheat was increased to 0%, 7%, 12% and 15% in the 4 feeding phases, deviating from Study 1, and also included in the ration planning (Table 3). All diets contained a phytase and xylanase, as commonly found in commercial feed mixes.

Table 3. Ingredients and calculated (N-analyzed) nutrient composition of the diets in Study 2.

	Starter	**Grower I**		**Grower II**		**Finisher**	
		Control diet (standard)	Experimental diet (N reduced)	Control diet (standard)	Experimental diet (N reduced)	Control diet (standard)	Experimental diet (N reduced)
Ingredients (%)							
Wheat-unground		7.0		12.0		15.0	
Wheat	23.8	30.4	34.4	33.4	41.9	28.8	37.6
Corn	36.0	24.0		20.0		20.0	
Bakery meal		2.8				2.6	
Soybean meal	25.5	25.0	20.8	22.1	13.2	19.5	10.4
Soybean concentrate	3.0						
Rapeseed full fat	3.0	2.0		2.0		2.0	
Rapeseed expeller	2.0	2.0		4.0		5.0	
Vegetable oil	2.1	3.1	2.5	3.2	2.0	4.5	3.2
Lysine sulfate 70	0.48	0.38	0.57	0.40	0.79	0.37	0.77
DL-Methionine	0.39	0.26	0.30	0.25	0.31	0.22	0.29
L-Threonine	0.15	0.09	0.21	0.08	0.28	0.09	0.27
Tryptophan			0.01		0.01		
L-Valine	0.10	0.06	0.14	0.07	0.20	0.05	0.21
L-Arginine			0.18		0.34		0.35
L-Isoleucine			0.11		0.20		0.21
Glycine			0.10		0.20		0.15
Minerals and Premix ^{a}	3.5	2.9	3.1	2.5	2.6	1.9	1.9
Energy (MJ AMEn/kg) and Nutrients (%)							
AMEn, MJ/kg	12.6	12.9		12.9		13.4	
Ether extract	5.9	6.6	6.0	6.5	5.2	8.1	6.8
Crude fiber	3.2	3.1	3.0	3.2	3.0	3.2	3.1
Ash	6.4	5.7	5.5	5.2	4.9	4.5	4.2
Starch	38.1	39.9	42.3	41.1	46.3	41.1	46.5
Crude protein—expected ^{b}	21.8	20.3	19.5	19.8	18.0	18.9	17.1
Raw protein—analyzed ^{c}	21.9	20.1	19.5	20.1	18.4	18.7	17.2
Lysine ^{d}	1.38	1.18	1.20	1.17	1.19	1.10	1.03
	(1.36)	(1.20)	(1.19)	(1.17)	(1.14)	(1.09)	(1.07)
Methionine + Cysteine ^{d}	1.02	0.82	0.87	0.86	0.78	0.76	0.81
	(1.06)	(0.90)	(0.90)	(0.89)	(0.86)	(0.84)	(0.82)
Threonine ^{d}	0.91	0.80	0.82	0.77	0.80	0.77	0.74
	(0.93)	(0.81)	(0.86)	(0.78)	(0.83)	(0.75)	(0.79)
Arginine ^{d}	1.35	1.22	1.28	1.19	1.26	1.12	1.17
	(1.37)	(1.25)	(1.31)	(1.20)	(1.28)	(1.14)	(1.22)
Valined	1.09	0.94	0.96	0.94	0.92	0.89	0.86
	(1.08)	(0.97)	(0.97)	(0.96)	(0.93)	(0.90)	(0.89)
Isoleucined	0.90	0.80	0.84	0.78	0.81	0.74	0.76
	(0.89)	(0.82)	(0.85)	(0.79)	(0.82)	(0.75)	(0.78)
Glycine $_{\text{equivalents}}$ ^{d,e}	1.61	1.47	1.47	1.45	1.41	1.37	1.28
	(1.61)	(1.51)	(1.48)	(1.47)	(1.40)	(1.41)	(1.28)
SID Lysine	1.23	1.08	1.08	1.05	1.05	0.98	0.98

Table 3. Cont.

	Starter	Grower I		Grower II		Finisher	
SID Methionine + Cysteine	0.97	0.81	0.82	0.80	0.79	0.76	0.76
SID Threonine	0.80	0.69	0.75	0.66	0.74	0.64	0.70
SID Arginine	1.24	1.13	1.20	1.09	1.18	1.02	1.12
SID Valine	0.96	0.86	0.87	0.85	0.84	0.79	0.81
SID Isoleucine	0.79	0.73	0.76	0.70	0.76	0.66	0.72
SID Glycine $_{\text{equivalents}}$	1.38	1.30	1.29	1.26	1.23	1.20	1.12

[a] containing phytase and xylanase, starter and grower diets contained coccidiostats [b] according to Kjehldahl [c] analyzed according to Dumas [d] analyzed values, in brackets: expected; analyses based on starter feed: $n = 7$; Growers I: $n = 14/16$; Growers II: $n = 12/8$; finisher feed: $n = 23/17$ for standard feed/experimental feed, respectively, analyzed values > 0.03%-points lower than expected in bold; [e] Gly $_{\text{equivalents}}$: Gly + 0.814 × Ser.

In both studies (Study 1 $n = 97$; Study 2 $n = 7$), each batch of feed was analyzed. The crude nutrients and entire amino acid profiles were analyzed according to the Commission Directive [17]. Amino acid concentrations (excluding tryptophan and tyrosine) were analyzed using ion exchange chromatography [17].

The higher-than-expected CP values can partly be explained by different N analysis methods. During optimization in the commercial feed mill, protein content resulting from the Kjeldahl process was used. The protein content of the 97 feed samples was determined using the Dumas method (reference method), which determines 100% of the N content through burning. With the Kjeldahl method, azo and nitro groups cannot be broken down and therefore cannot be determined. According to Müller and Foss [18], 2% of the Dumas protein cannot be detected using Kjeldahl. Deducting this 2% would thus explain a large portion of the difference in Study 1.

2.3. Data and Sample Collection

Study 1: Data collection took place at the barn level (5 replicates per variant) and at the sample level (50 animals per house). Water intake and mortality were automatically recorded for the entire fattening period in each barn. The feed intake per barn was determined from the delivered batches of feed minus the leftovers, while the consumption per animal was calculated from the corresponding number of animals delivered to the slaughterhouse. The feed conversion resulted from the ratio of the feed delivered (minus the weighed leftover feed) and the live weight delivered to slaughter. The reports of the data collected at the slaughterhouse allowed for the number of delivered animals, the live weight (kg) and a footpad score to be evaluated for each slaughter date and barn. The footpads were classified by a camera system: 0 (slight lesions), 1, 2a and 2b (severe lesions) (Table 4). Reporting of slaughter and carcass data was not possible. In order to be able to better assess the weight development during fattening, the live weight (g) and a footpad rating were determined for 50 animals per barn on days 0, 10, 22, 28 and 38. The worse footpad was evaluated for each animal. The evaluation was always carried out by the same trained person (Table 4).

The selection of the 50 animals took place without sex determination at 4 places in the barn—after almost a third and two thirds of the barn length, animals were selected from the left main passage, after just over a third and just over two thirds from the right main passage (Figure 1).

The selection of the animals, therefore, extended to 4 different locations in relation to the barn length. At each point, 12–13 chickens were randomly selected within a radius of about 5–6 m. On days 0, 10, 22, 28 and 38, the dry matter (DM) content in the litter of all houses in Study 1 was determined. For this purpose, a representative mixed sample was formed from 4 individual samples. The individual samples were taken from the floor area at a considerable distance from the feeding and drinking lanes (Figure 1). On day 38, additional litter samples were taken to analyze their nutrient content by removing a core sample from the litter down to the concrete floor across the entire width of the barn after about 25%, 50% and 75% of the barn length. The areas of habitation, trough and drinking were sampled separately (Figure 1). Homogeneous mixed samples for the analysis were formed from the core samples separately for the 3 areas. After emptying the barn, the amount of litter from each barn was weighed with a weighing device on the front loader of the tractor.

Table 4. Evaluation scheme of the ball of the foot changed according to Hocking et al. (2008) [19] and at the slaughterhouse using camera classification.

Score	According to Hocking et al. (2008) [19]	Score	Camera Classification Slaughterhouse
0	No outward signs of foot pad dermatitis (FPD). Skin feels soft. Lesions and necrosis are not present.		
1	Ball of foot feels harder and more compact. Net-like scales are (slightly) differentiated or separated from each other. Small black necrosis visible.	0	1–5% necrotic area of footpad
2	Ball of foot is noticeably swollen. Reticular scales well-differentiated and scale-like necrosis developed and visible. White scar tissue partially around necrotized area. Necrotic area ≤1/4 of the ball of the foot.	1	6–20% necrotic area of footpad
3	Ball of foot is severely swollen and enlarged. Numerous, distinct, single reticular scales. Necrotic area ≤1/2 of the ball of the foot.	2A	21–50% necrotic area of footpad
4	Matches Description Score 3. Except: Necrotic area >1/2 of ball of foot	2 B	>51% necrotic area of footpad

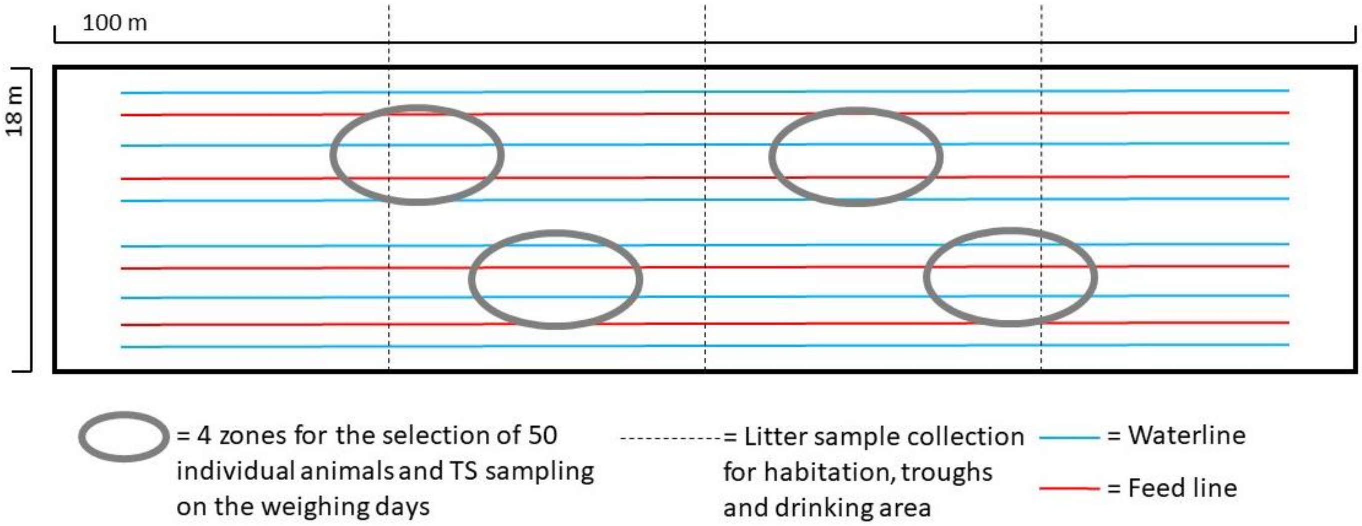

Figure 1. Sampling scheme in the barns for the selection of the individual animals, dry matter samples on the weighing days and litter samples at the end of fattening.

Study 2: Data collection for the parameters' feed intake, feed conversion, litter quantity and litter quality took place per box (*n* = 250 animals, 5 repetitions). On days 0, 10, 29 and 34, the live weights of 50 animals per pen were recorded at the individual animal level and a footpad rating was carried out (according to Hocking et al. [19], Table 4). The feed consumption was recorded per feeding phase per compartment.

In Study 2, a mixed sample was also taken from each box after emptying the pen in order to determine the nutrient content in the litter. The litter quantities per variant (not per compartment) were then weighed directly. In the litter samples from both studies, the N content in the original substance, the dry substance content and the N content in the dry matter were determined using NIR (DIN ISO 11261;1997-05 and DIN EN 12880-S 2a;2001-02). However, it should be noted that bedding material was added during the course of the experiment.

An N balance was calculated from the data obtained:

- N Intake (kg/barn) = feed consumed (kg) × analyzed N-content (%);
- N deposition (kg/barn) = kg live weight delivered to the slaughterhouse × 30 g N/kg live weight [20];
- N Excretion (kg/barn) = N Intake—N Deposition;
- N Utilization (%) = N Deposition (kg/barn)/N Intake (kg/barn) × 100.

The European Efficiency Index (EEI) was used as a productivity index based on biological performance.

$$\text{EEI} = ([\text{Survival rate}, \% \times \text{final body weight, kg}]/[\text{FCR} \times \text{age, d}] \times 100)$$

2.4. Statistical Analysis

The dry matter content in the litter and the manually recorded data from Study 1 (large-scale experiment) as well as all data from Study 2 (pen facility) were calculated using the statistical program package IBM SPSS Statistics for Windows, version 24.0. For the manual weight recording and the footpad scores, the individual animal served as the experimental unit. The barn (Study 1) or pen (Study 2) served as the experimental unit for the feed intake, the slaughter data, the litter moisture and the N calculations. The program was also used for the manual scoring of the footpad and the Mann–Whitney U test was used to check for differences at each recording time. The barn-related data of the commercial study were determined using the T test evaluated as a comparison of mean values. Significant treatment differences were reported at $p \leq 0.05$, statistical trends at $p \leq 0.1$.

3. Results and Discussion

3.1. Feed Analysis

When calculating the weighted average, it becomes clear that a higher reduction in the finisher phase has the greatest effect, because the amount of feed increases significantly with each phase and is therefore more important in the calculation of the weighted average. The reductions were achieved in particular by adjusting the three macro-components of soybean meal, vegetable oil and wheat. Accordingly, the weighted average soybean meal content was reduced by 9% from 204 kg/t to 185 kg/t in Study 1 and by 34% from 221 g/t to 146 g/t in Study 2. While Selle et al. [21] reported potential savings of soy of up to 60% in semi-synthetic test feeds, it should be mentioned here that in Study 1, peas, forming an average of 7.3% of the total feed mass, already accounted for around 1.5% of the feed protein. In addition, the protein decreases in the experiments were relatively small compared to the studies referenced by Selle et al. [21]. However, the present study shows that even small changes in the feed specifications had a significant impact on the use rates of the protein sources.

Each batch of feed in Study 1 was analyzed ($n = 97$). The analyzed amino acid (AA) contents showed very good agreement with the calculated values (Table 2, analyzed values in brackets). In addition, there was only a small variation in the amino acid concentration within the treatments and the phases, which was a maximum of 5% and in most cases was below 2%. This homogeneity demonstrates that a commercial feed mill can mix very precisely, with a prior accurate raw material evaluation being a crucial requirement. The analyses of the experimental diets of Study 2 ($n = 15$) were each based on only one batch per phase and treatment. Somewhat greater deviations from the expected values were observed here, especially in the low-protein diets. The Thr should be particularly emphasized here, which, according to recent scientific findings of Chrystal et al. and Star et al. [16,22], should be raised above the level of the corresponding standard feed. However, the feed analysis could only identify approx. 80% of the planned threonine addition. The recovery rate of the planned lysine (Lys) and arginine (Arg) was also below our expectations. Since these deviations were found in the finisher feed in particular, an influence on the performance cannot be ruled out.

With regard to protein content, there were slight differences between expected and determined values, particularly in Study 1. These were partly based on the differences between the analytical models, calculated (Kjehldal) and analyzed N, (Dumas) described above. The maximum CP deviation was 0.7 percentage points above the calculated value. In the test group, taking into account the total feed intake, this led to a lower average protein supply of 0.3 percentage points instead of a calculated difference of 0.5 percentage points. The analyzed CP levels in the N-reduced variant resulted in an average of 19.6% CP, weighted proportionately to the feeding phases, in contrast to 19.9% in the standard feed in Study 1, while the levels in Study 2 were 19.9% and 18.4%, respectively (Table 3). The total N content is made up of amino acid N and non-protein N (NPN), with the latter contributing a relatively constant 10% to the rations used. Only the amino acid N can potentially be retained by the animals in endogenous proteins, while NPN is mainly metabolized and excreted [23,24]. In addition, ration optimization based on the nitrogen bound in AA would significantly improve the accuracy of the calculation. Since the NPN fraction is mostly excreted and also varies greatly in different batches of the individual components, this makes a calculation based on CP very imprecise if the fraction that is certainly excreted is unknown [25]. Nevertheless, the analyzed N contents were used for the N balance calculations and not the planned N contents while the analysed AA levels precisely reflected expectations, particularly for Study 1.

3.2. Broiler Performance

The house-related performances of the broilers from Study 1 are shown in Table 5. The live weights according to the slaughterhouse report, both individually for grips 1, 2 and the main grip, as well as for the overall average, did not differ between the treatments ($p \geq 0.05$).

The main grip on days 40–42 showed equal growth performance ($p = 0.880$) with the N-reduced variant. Random sampling of 50 animals at different times per pen (Table 6) were confirmed to be similar for both treatments on days 0, 10 and 22. According to this, the birds in the experimental group developed slightly more slowly and weighed about 20 g less on day 28 and about 29 g less on day 38 than the animals in the control group, but the course of growth was far from being statistically verified ($p = 0.835$). It should be noted that a random sample of 50 animals per barn represented only around 0.1–0.2% of the barn population, and a comparison of day 28 manual body weights with day 29 weights of first thinning, as well as day 38 day manual body weights with the overall average body weights with day 37 confirmed a reasonable agreement. The advantage of manual data collection, however, is that it is then possible to evaluate the course of growth. With a difference of 9 g at the slaughterhouse, more than 23,000 animals per barn were included in the data set, so this value is far more robust.

Well-optimized rations with similar protein contents also led to identical increases despite protein reduction in other studies [10,16,26–30]. An analyzed average CP content of 17.2% CP (experimental diet) in the finisher diet and an optimal AA profile up to and including arginine and isoleucine allows for the same performance compared to the standard diet (18.8% CP in the finisher). Hilliar et al. [31] also showed that with 16.7% CP in the final fattening and AA balancing up to arginine and isoleucine, no performance losses occurred.

Despite the relatively high reduction in protein in Study 2, the weight development of the low-protein variant over the course of the entire experiment was also equivalent to that of the control group (Table 7).

This confirmed once again that well-optimized dietary protein (including arginine and isoleucine, increased threonine and glycine levels and a protein reduction of up to approx. 16.5–17% CP in the finisher feed) do not necessarily lead to any reduced performance. The performances in Study 2 were even more astonishing since the protein reduction was more pronounced and the animals in the small experimental pens were at a significantly higher performance level. Despite stronger N reductions, the results were on the same level as those of Lemme et al. [32], which were collected in the same test facility. If the performances in the commercial area (Study 1) were converted to the identical weighing day (day 34), the live weight would be approx. 300 g below the performances in the pens in Study 2.

Table 5. Performance of 420,000 mixed-sex broilers fed commercial standard (210,000 birds, $n = 5$) or N-reduced (210,000 birds, $n = 5$) feeds over the entire production cycle.

	Control Diet	**Experimental Diet**	**Pooled SEM [d]**	***p* Values**
	standard	N-reduced		
Body weights, kg/bird				
Grip 1, day 29	1.550	1.523	0.0316	0.366
Grip 2, day 34	2.016	2.004	0.0280	0.638
Main grip, days 40–42	2.789	2.780	0.0603	0.880
Overall results				
Average age, days [a]	36.7	36.7	0.333	0.943
Body weight—overall, kg/bird [a]	2.344	2.328	0.0425	0.685
Feed intake—overall, kg/bird [a]	3.547	3.463	0.0474	0.083
Feed per gain, kg/kg [a]	1.517	1.496	0.0144	0.138
European efficiency index [b]	415	419	8.7	0.611
Mortality, %	1.14	1.36	0.06	0.022
Feed intake—kg/barn				
Starter	10,502	10,336	57	0.091
Growers 1	33,051	33,933	300	0.129
Grower 2	30,216	30,768	213	0.057
Finishers	73,067 [a]	67,608 [b]	1078	0.003
Water and litter				
Water intake, ml/bird	7.789 [a]	7.476 [b]	135.0	0.032
Litter weight, t/barn	54.6	47.9	4.67	0.148
N balance, kg/barn				
N intake	4666 [a]	4464 [b]	57.6	0.004
N deposition [c]	2852	2810	51.9	0.393
N excretion	1814 [a]	1654 [b]	8/31	<0.001
N-Utilization, % of intake [c]	61.1 [a]	62.9 [b]	0.65	0.014

[a] Weighted average including all crops; [b] European Efficiency Index = ([Survival rate, % × final body weight, kg]/[FCR × age, d] × 100); [c] assuming 30 g N/kg final body weight [22]; [d] SEM: standard error of means, five observations per treatment mean, different letters indicate significant differences ($p \leq 0.05$); statistical tendency: $p \leq 0.1$.

Table 6. Development of the live weights (in g) of broilers when offered a standard or protein-reduced complete feed in Study 1.

	Control Diet, Standard (*n* = 250)		**Experimental Diet, N-Reduced (*n* = 250)**	
	(g/bird)	Sd	(g/bird)	sd
Day 0	41	(±3)	41	(±3)
Day 10	287	(±29)	291	(±29)
Day 22	1050	(±120)	1050	(±115)
Day 28	1504	(±171)	1484	(±181)
Day 38	2466	(±314)	2437	(±298)

50 birds per house in 5 houses; SD = standard deviation.

3.3. Feed Intake

The animals in the experimental group in Study 1 tended to eat less feed (3.46 kg/animal) compared to the control group (3.55 kg/animal) ($p = 0.083$). This difference resulted solely from the last phase of fattening (Table 5), in which the feed consumption was significantly different ($p = 0.003$). In the first 3 phases, the feed consumption per barn was almost identical between treatments. Over the entire Study 1, the test group consumed an average of 5.4 t less than the control group (Table 5) and the same growth rates resulted at the end of fattening. In Study 2, identical feed intakes were recorded for the two variants. Similar observations were made in other trials, including significantly lower feed intake when low-protein feed was offered [33]. Whether the reason for this was a higher feed conversion in general, a higher N conversion or a lower metabolic load cannot be clearly determined based on the available data. In contrast, there are also a large number of experiments in which feed intake was not influenced by the protein reduction [10,16,29,32,34].

Table 7. Performance of 2500 mixed-sex broilers fed commercial standard (n = 5 pens) or N-reduced (n = 5 pens) feeds over the entire production cycle of Study 2.

	Control Diet	Experimental Diet	Pooled SEM [c]	*p*-Values
Body Weights, g/bird [a]	**Standard**	**N-Reduced**		
Day 0	42.6	42.7	0.45	0.810
Day 10	326	324	1.8	0.268
Day 29	1830	1839	33.0	0.779
Day 34	2403	2392	22.9	0.601
Body weight gain, g/bird/phase				
Starter	284	282	1.8	0.222
Grower	1504	1515	33.1	0.730
Finisher	573	553	27.7	0.448
Feed conversion ratio, kg/kg				
Overalls	1443	1439	0.014	0.810
Nitrogen balance, g/bird				
N intake	110.7 ^{a}	101.7 ^{b}	0.10	<0.01
N deposition [b]	70.6 ^{a}	70.5	0.25	0.461
N excretion	40.1 ^{a}	31.7 ^{b}	0.20	<0.01
Retained of ingested N [b]	63.8 ^{b}	69.3 ^{a}	0.19	<0.01
Retained of ingested potentially available N	75.7 ^{b}	82.2 ^{a}	0.22	<0.01

[a] 250 birds, 50 birds per pen; [b] assuming 30 g N/kg final body weight [20]; [c] SEM: standard error of means, 5 observations per treatment mean, different letters indicate significant differences ($p \leq 0.05$); statistical tendency: $p \leq 0.1$.

3.4. Feed Conversion

The average feed conversion ratio (FCR) in the test group of Study 1 was 1.496, where in the control group it was 1.517 (Table 5). A level of 1.5 in feed conversion in a commercial barn is considered very efficient. The average feed conversion of all farms in the federal state of Lower Saxony, a representative region for conventional chicken fattening, which took part in the business branch evaluation of the Lower Saxony Chamber of Agriculture for the 2019/20 marketing year, was 1.54 [35]. In contrast, a feed conversion ratio of approx. 1.44 in both variants in Study 2 is extremely efficient and showed what is possible under optimal conditions with greatly reduced but optimally balanced AA content. In many studies on protein lowering, FCR was also slightly improved or at an identical level compared to the control group [16,28,32,34,36,37]. It is striking that the CP reductions were in the range of 17–18% CP in the finisher phase and meticulous AA balancing took place, which makes the results of the present study comparable. It is noteworthy that the present Study 1, which was carried out on a very good commercial farm in terms of the performance parameters (e.g., approx. 68 g daily gain over the entire fattening period and 1.5 FCR), when compared with the numerous studies conducted in research facilities [16,29], was at the same level. Individual studies [29] have even shown a significantly lower FCR. Chrystal et al. [36] only started with the finisher phase on day 28, compared to Van Harn et al. [29]. Both used 3 feeding phases, but Van Harn et al. [29] defined these differently over the entire fattening period, which could explain the significantly improved FCR. Very strong CP reductions may also lead to poorer FCR if the latest findings on threonine and glycine nutrition are not taken into account [16]. In the finisher phase of the experimental group, the CP content was, for example, 15% and 16% CP [26], 17.57% CP [38], at 16% CP [39], at 16.25% CP [33], 15% [40], 15.5% CP [27]. This suggests that a protein reduction in the range of 16% poses an efficiency risk even with optimized AA content. Apparently, certain AA or some AA in combination are deficient in this area, for which there is still no scientific knowledge [26,36,38]. In part, however, the underperformance can also be explained by a lack of good professional practice with respect to phase feeding. For example, feeding a grower diet from day 14 to 35 to broilers may not meet the needs of a broiler on each day of growth, and therefore the FCR increases [39].

Application of a strong reduction in CP, like in Study 2, into commercial practice should be done in small steps. With stronger reductions in CP, maybe a higher number of feeding phases (e.g., 5 phases) or multi-phase reduction concepts are important in order to bring the feed optimization even closer to the bird's requirements during the production cycle, and thus maintain performance. In commercial studies, researchers will still have to take the approach of small reduction steps in order to avoid growth performance and FCR impairments, which occurred in many station studies in the range of 16% CP in the finisher phase. Since these diets are formulated very closely to the actual broiler requirements, inferior feed optimization or mixing inaccuracies in the factory can lead to a drop in performance and health. These risks can therefore be minimized through optimal feed and feeding management from the evaluation of the raw materials in the feed factory [41,42] to the exact feed presentation in the barn [43].

The EEI in Study 1, the experiment group (419) was at a similar level of the control group (415). The potential effects of protein reduction on growth performance, age in days, mortality and FCR also influence the EEI. However, slight variations in considered parameters can cancel each other out, so that the EEI is not changed that much by the protein reduction. In addition, effects on slaughter performance, especially the proportion of breast meat, were not taken into account. Thus, EEI should be seen as a productivity index rather than an economic indicator. However, it often indicates economical trends.

All comparable low-protein studies took place in small pens in special test facilities with optimal hygienic and management conditions. Due to different final ages, and thus, different final body weights and FCR, the EEI in other protein reduction studies were significantly higher (e.g., 471/454 [28] and 413 to 447 [29]) than in the present commercial study (Study 1). With 250 animals per compartment, the production conditions in Study 2 were much closer in practice to the usual station tests with, for example, 42 animals per compartment. Nevertheless, Study 2 achieved comparable or higher EEI values than the current literature on station tests shown. Both the standard and the low-protein variants had an EEI of 472.

3.5. Water Absorption and Litter Moisture

Excess nitrogen is converted in the liver into uric acid, which is excreted in the kidneys. This metabolic process causes an increased water requirement [44]. Small-scale feeding trials have often proven this relationship [10,32,40,45,46], so a similar effect on a commercial scale was expected. The fact that the animals in the large-scale test group consumed 4% less water ($p \leq 0.05$) confirms that less water was required for the excretion of excess nitrogen as uric acid. In addition, the N-reduced diets also contained less potassium because the potassium-rich soybean meal content was also reduced. This speaks for a further reduction of water requirement because the electrolyte balance was also reduced [47].

In the test group, the lower water consumption led to numerically drier litter in all areas in the barn (Table 8). Although an attempt was made to obtain samples that were as representative as possible using a sampling scheme, the variation between the areas in the barn was large, especially between the functional areas of feed intake, water intake and habitation, so that no statistical overall differences could be found. In addition, quantification of the litter according to the functional areas is not possible.

3.6. Footpad Health

Moist litter [48–50] and increased ammonia/pH levels negatively impact broiler footpad health [51,52] and thus decrease animal welfare. In Study 1, the results of footpad assessments at the slaughterhouse showed a very high footpad health status. With standard diets, an already very good footpad lesion score was achieved. Grades 0 and 1 covered almost 100% of all feet which scored at all 3 slaughter dates. However, using the level 0 (no changes) as a baseline, it is noticeable that the footpad health of the standard feeding group (94%, 89%, 82%) decreased over the course of the 3 slaughter dates and the footpad health of the test group remained very constant (95%, 99%, 98%). The biggest difference between treatments was, therefore, at the main harvest. The difference in the assessment grade 0 was at a p-level of $p \leq 0.102$ and in the assessment grade 1 at $p \leq 0.091$ and indicated a tendency. The results of the manual footpad assessment (Table 9) also showed a continuous deterioration of the footpads in the control group with age. In contrast, analogous to the camera classification at the slaughterhouse, the test group showed $\geq$ 95% healthy footpads (rating 0) up to the last rating on day 38. In comparison, only 70% of the footpads in the control group was rated 0 on day 38. This difference on day 38 was significantly verified ($p \leq 0.032$). In addition, on days 22 and 28 there was a statistical tendency for healthier footpads in the test group. Despite the beneficial effects of dietary protein reduction, footpad scores were generally at a very good level. In poorly managed barns there should be a significantly greater potential for improving footpad health by protein reduction. It is also noteworthy that these results were already achieved with a moderate protein reduction of only 0.3% (weighted average).

Table 8. Results of the mean dry matter content of the litter of the control and experimental variant on trial days 0, 10, 22, 28, 38 in Study 1.

Litter Dry Matter (%)	Control Diet, Standard	Experimental Diet, N-Reduced	SEM	p
Day 0	90.19	90.79		0.572
Day 10	74.32	75.09		0.723
Day 22	67.11	67.94		0.793
Day 28	61.02	64.16		0.294
Day 38	56.23	58.52		0.331

Table 9. Percentage distribution of the scores of the manual footpad scoring in the barns on the days of data collection per variant in the commercial feeding trial based on 50 randomly selected birds per barn (Study 1).

Day	Treatment	Percentage of the Rating (%)					
		0	1	2	3	4	p
0	1	100.0	0.0	0.0	0.0	0.0	1000
	2	100.0	0.0	0.0	0.0	0.0	
10	1	96.4	2.8	0.8	0.0	0.0	0.421
	2	98.4	1.6	0.0	0.0	0.0	
22	1	91.6	4.4	2.4	1.2	0.4	0.056
	2	98.4	0.8	0.0	0.8	0.0	
28	1	83.6	6.4	8.0	1.6	0.4	0.095
	2	98.4	0.8	0.4	0.4	0.0	
38	1^{a}	69.6	6.0	15.6	6.8	2.0	0.032
	2^{b}	94.8	1.2	3.6	0.4	0.0	

Treatment 1 = standard; Treatment 2 = N-reduced, different letters indicate significant differences between variant per day ($p \leq 0.05$); statistical tendency: $p \leq 0.1$.

In studies by Li et al. and Nagaraj et al. [53,54], the occurrence and severity of podo dermatitis could also be reduced by lowering the protein level in the feed. Lemme et al. [34] were also able to prove that the overall grades were significantly improved under German conditions as a result of protein reduction. As part of the increasing efforts to improve animal welfare in Germany, the improvements in footpad health are considered important as repeatedly reported poor footpad health may result in certain interventions on farms, which may include an obligatory reduction of stocking density.

In Study 2, almost all ratings were rated 0 up to the end of the study, since bedding material was added to individual pens several times if necessary, thereby creating optimal conditions for both feed variants. It is all the more astonishing that the dry matter content at the end of the examination on day 34 was 12 percentage points higher in the test group (62% dry matter) than in the control group (50% dry matter).

3.7. Amount of Litter and Environment/N Balance

The moderate reduction of the CP by 0.3 percentage points in Study 1 and 1.5 percentage points in Study 2 led to the same average daily gain, as well as to a quantitatively and qualitatively lower N excretion with consequences on the N balance (Table 7). The N uptake could be reduced by 4% ($p \leq 0.05$) and the calculated N excretion by 9% ($p \leq 0.05$). If this causal relationship were extrapolated linearly, a CP reduction of 1 percentage point in future studies would result in the potential for a reduction in N excretion of approx. 22% (Study 1) and approx. 18% in Study 2. Van Harn et al. [29] calculated a saving in N excretion of only 3.34% per percentage point of N reduction. Santonja et al. [1] presented approx. 10% lower N excretions, and Lemme et al. [34] around 15%, 14% and 12% reductions in N excretion per percentage point CP reduction in the feed. In summary, with a few exceptions, at least 10% less N excretion per percentage point of CP reduction can be assumed per percentage point CP reduction.

The calculated N excretion (feed analysis x feed amount minus deposition, Table 5) were confirmed in litter analyses and the recording of the litter weights (Tables 5, 10 and 11). Slightly reduced N content in the organic substance and increased dry matter content led to significant N savings in litter dry matter in relation to the area "habitation" (5.5% Reduction) in Study 1. In the vicinity of the trough, the reduction is 3.1 %. Due to the very homogeneous values measured, the T test showed significance for the trough area ($p \leq 0.029$). The different DM and N contents in the habitation area for trough and drinking illustrate the problem of a representative nutrient analysis in the litter. Sampling after removing all animals by creating a composite sample using individual samples from the litter heap from the entire barn that had been pushed together does not guarantee a representative, homogeneous composite sample either. Exact determination of the weight proportions of the area's habitation, trough and drinking trough for the percentage calculation of the analysis results would be useful, but means a high amount of effort.

Table 10. Mean N content of the original substance and the dry matter of the different zones in the barn in Study 1 (average, $n = 5$ per variant).

Parameter	Treatments	Habitation Zone	SEM	*p*-Value	Drinking Zone	SEM	*p*-Value	Trough Zone	SEM	*p*-Value
N in litter (%)	1	3.01	0.039	0.726	3.17	0.072	0.282	3.74	0.037	0.584
	2	2.97			3.35			3.69		
DM content (%)	1	56.87	1.215	0.359	62.08	1.911	0.260	76.59	0.811	2.564
	2	59.58			67.25			78.17		
N in DM (%)	1	5.29	0.087	0.130	5.13	0.073	0.381	4.88 ^{a}	0.036	0.029
	2	5.00			4.99			4.73 ^{b}		

Treatment 1 = standard; Treatment 2 = N-reduced, different letters indicate significant differences ($p \leq 0.05$); statistical tendency: $p \leq 0.1$.

Table 11. Amount of litter and average litter quality in the Haus Düsse trial per variant (Study 2).

Variant	Control	Low Protein
Amount of litter (kg)	2120.00	1460.00
DM content (%)	50.02	61.92
DM litter (kg)	1060.42	904.03
N (% of TS)	4.56	3.89
N amount (kg)	48.40	35.17
% decrease		−27.34

The weighed amount of litter per barn in Study 1 (Table 5) was 54.6 t in the control group and 47.9 t in the test group, and thus corresponded to a reduction in litter of approx. 12.3%. The amounts of litter multiplied by the N content of the original substance in the area from Table 10 resulted in an N accumulation per barn of 1643.5 kg N in the control group and 1422.6 kg N in the test group. Subtracting 40% of the losses during storage and application [4] with a maximum application of 170 kg N from organic origin per hectare (EU-Level) [55] resulted in an area requirement per barn and flock of 5.79 ha in the control group and 5.05 ha in the experimental group (−12.84%). In Study 2, the amount of litter was also significantly reduced (−31.1%). This effect was mainly due to the lower water content, despite additional bedding during the experiment. The high CP reduction of 1.5% protein showed its effects in the parameters of the litter (Table 11). Even with the standardization of the litter for dry matter in kg, significant savings were still made (−14.75%). Additionally, due to a 14.7% lower N content in the litter, the percentage decrease in the amount of N and thus the reduction in land required for application was 27.3%.

The indirect N balance resulted from the analyzed raw feed protein content, the corresponding feed consumption and the delivered live weight multiplied by 30 g N/kg live weight [20]. Accordingly, 1814 kg N (standard) or 1654 kg N excretion (experimental) would accrue per barn, that is, 10.6% or 15.7% higher values than the values determined via the litter analyses. For Study 2, 48.8 kg and 38.0 kg N excretion per pen were calculated, which are 1% and 8% higher than the values determined via litter analyses (Table 11). The assumption of 30 g N/kg LW for broilers was recently reconfirmed, suggesting that the indirect method is more reliable. Part of the observed differences between the methodological approaches can be explained by discharges as ammonia. Lemme et al. [34] reported that NH_4 accounted for about 15% to 22% of the total N measured in litter. Interestingly, this relative proportion decreased with diet protein reduction. Belloir et al. [26] also reported decreasing N volatilization with feed protein reduction in the finisher feed. Correspondingly, the losses ranged from about 12% with low feed protein content and up to 36% with standard protein content, which confirms the differences from this study of 11.6% and 15.7%, respectively. Hernandez et al. [46] also reported increasing cumulative NH_3 losses determined in commercial farms in the finisher phase and observed significantly lower NH_3 losses with feed protein reduction. Due to these imponderables, companies in Germany are indirectly balanced according to the legislation [4]. The present results showed that even small reductions in the N content of broiler feeds can significantly reduce the necessary and land-related application of N, and thus the N balance of commercial farms could be improved.

A lower N intake ($p \leq 0.05$) and an almost identical live weight with 30 g N/kg LW resulted in a significantly improved N utilization ($p = 0.014$) in Study 1. The values of the control group (61.1% N-utilization) and the experimental group (62.9% N-utilization) in Study 1 were at a very high level for commercial conditions. Nevertheless, the practical test showed that significantly better N utilization can also be achieved with moderate N reduction. DVT [6] indicated that about 313 g CP (50.1 g N) was needed to produce 1 kg live weight (30 g N) in practice in 2020. The current practice in Germany therefore has 59.9% of N utilization and is thus slightly below the values in Study 1. In the smaller station pens in Study 2, N efficiencies of 63.8% were determined with standard feed and 69.3% in the heavily N-reduced variant ($p < 0.05$). This shows the efficiency potential for commercial practice, as well as the efficiency potential for further N reductions. Other studies in smaller facilities also showed N utilization of over 69% [16]. Belloir et al. [26] modeled the relationship between feed protein content and N utilization in the finisher phase (21–35 days, male broilers) and came up with values of around 72% with an extremely low 15% RP. While animal performance also decreased there, the regression coefficients suggested that utilization would change by 3.2 to 3.6 percentage points for each percentage point protein reduction. For the extrapolation for Study 2, which includes the entire fattening period, an increase in N utilization of 3.7 percentage points per percentage point for CP reduction can be determined, while Study 1 would even suggest 6 percentage points are possible.

The large savings potential in N excretion in both studies and the comparative studies mentioned also suggest a reduction in gaseous losses [26]. The targets set at national level via the Material Flow Balance Ordinance and Fertilizer Ordinance [2–4] and at European level with the EU-NEC Directive 2016/2284 [5] and a savings target of 29% ammonia are realistic in Germany.

3.8. Slaughter Data

The commercial slaughterhouse in Study 1 could not provide any data that could be clearly assigned to the treatments. However, the commercial farmer has not received any performance differences from the slaughterhouse in this regard, so that only the live weight of the animals was taken into account when paying, and the carcass must have met the standard. Van Harn et al. and Lemme et al. [29,34] showed that with protein reduction to a very similar protein level in the final fattening (16.8% CP) as the present studies, the partial weight of the breast can be reduced. In contrast, Lemme et al. [32] could not detect a decrease in the proportion of breast meat up to 17.5% CP in the final fattening and Belloir et al. [26] up to 16% CP, especially since the findings of Chrystal et al. [16] in relation to increased threonine and glycine content were not taken into account.

Study 2 clearly showed that a sharp protein reduction to 17% CP in finisher feed in the semi-commercial range works when looking at overall gain. In the present study, it must be kept in mind that in the N-reduced variant, especially the finisher feed, contains only about 80% of the planned lysine and threonine supplementation. The undersupply may have limited the meat production potential because threonine and lysine are very important for meat development [56]. Muscle development, in particular, reacts sensitively to the supply of AA and even small deviations can be noticeable here, while overall growth and feed conversion were not affected. The proportion of breast in the entire body increases significantly with increasing body weight. For example, a chicken weighing 1.2 kg has around 18% breast meat. A chicken weighing 2.8 kg has about 25% breast meat (+7% percentage points relative to body weight). During this period, the legs only gain approx. 1.5% percentage points relative to the body weight. The wings even lose 0.25% relative to the whole body weight during this period [57]. Müsse et al. [58] were also able to determine the change in the proportion of the parts with increasing age.

Because the proportion of muscle tissue in the total weight gain increases during the course of fattening, the N content per kg of gain also increases. This results in changed requirements of the various AA (Lys, Thr). Pack et al. [59] reported from comparative meta-analyses that higher contents of Lys and Thr in the feed were necessary to optimize breast meat production than necessary to maximize overall gain or feed conversion. It can be assumed that not only can growth performance be maintained at a 1.5% CP reduction, but also slaughter performance if the increased threonine supply and the conditions of protein reduction have been sufficiently met [16].

4. Conclusions

A moderate reduction in dietary CP by 0.3% of the total feed consumed in Study 1 did not have any negative effect on performance in commercial broiler fattening if an adequate supply of essential AA, according to the latest scientific knowledge, is ensured. Similar advantages of a reduced protein supply could also be achieved in the second study with five-fold higher CP reduction: the same growth performance and feed conversion, drier litter, healthier footpads, less litter weight, less N-excretion, and thus, improved N-efficiency. It is emphasized that performance and N-utilization were already at high levels with commercial standard feed. In this way, compound feed companies and commercial broiler farmers can use the available results as a basis for N reduction in common feed mixtures without risking reduced performance. A special feature in commercial research, in addition to health pressure, the high number of animals, etc., is the mixed-sex housing. In Brazil, for example, fattening in the commercial sector is done with single-sex groups. This enables more precise feeding directed to the needs of each sex. In contrast, mixed-sex fattening in Germany requires certain compromises at the expense of utilization rates. Larger differences in the live weight of the different sexes occur, especially in the last 10 days of fattening. For example, the breeding organization for Ross 308 at day 40 reports the cock (2.918 g LW) to be 390 g heavier than the hen (2.528 g LW). In Study 2, a weighted average protein content of 18.4% CP, well below the current CP content in practice, was fed to the birds, and the same growth performance was achieved compared to a control group with 19.9% CP. With 250 animals per pen, the investigation in the research facility can simulate practice better than facilities with very small pens. It was clearly shown here that with a greater CP reduction without loss of growth, there is a lot of potential for greater N reductions (Study 2 with 27.3% reduction in N excretion) in commercial farms.

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Institutional Review Board Statement: The study did not include procedures to animals of a type that requires formal approval from an animal ethics committee because the whole data collection, except the recording of the live body weight, took place after slaughter and all animals were slaughtered for the use in the food chain. Recording the body weight of broilers is considered as good practice in management of broiler flocks. All authors confirm that any aspect of the work covered in this manuscript that has involved animals has been conducted according to the Guide for the Care and Use of Agricultural Animals in Research and Teaching, fourth edition, 2020, published by the American Dairy Science Association, the American Society of Animal Science, and the Poultry Science Association. All animals used were housed according to the German Order on the Protection of Animals and the Keeping of Production Animals prior to slaughter. Sampling procedures were in accordance with the German Animal Welfare Act, the regulations on the welfare of animals used for experiments or for other scientific purposes and national and international regulations on the welfare of animals at slaughter (Council Regulation (EC) No. 1099/2009, of 24 September 2009, on the protection of animals at the time of killing).

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