agriculture

MDPI

Article

Effect of Feeding Low Protein Diets on the Production Traits and the Nitrogen Composition of Excreta of Broiler Chickens

Nikoletta Such [1], László Pál [1], Patrik Strifler [1], Boglárka Horváth [2], Ilona Anna Koltay [1], Mohamed Ali Rawash [1], Valéria Farkas [1], Ákos Mezőlaki [3], László Wágner [1] and Károly Dublecz [1,*]

[1] Institute of Physiology and Nutrition, Georgikon Campus, Hungarian University of Agriculture and Life Sciences, Deák Ferenc Street 16, 8360 Keszthely, Hungary; such.nikoletta.amanda@phd.uni-mate.hu (N.S.); pal.laszlo@uni-mate.hu (L.P.); strifler.patrik@phd.uni-mate.hu (P.S.); koltay.ilona.anna@phd.uni-mate.hu (I.A.K.); mohamedali@post.agr.cu.edu.eg (M.A.R.); farkas.valeria@uni-mate.hu (V.F.); wagner.laszlo@uni-mate.hu (L.W.)

[2] UBM Feed Zrt., Kisvasút Street 1, 2085 Pilisvörösvár, Hungary; boglarka.horvath@ubm.hu

[3] Agrofeed Ltd., Duna Kapu Square 10, 9022 Győr, Hungary; akos.mezolaki@agrofeed.hu

* Correspondence: dublecz.karoly@uni-mate.hu

Abstract: The main goal of the current study was to investigate the effects of feeding low protein (LP) diets on the performance parameters and excreta composition of broiler chickens. In total, 288 male Ross 308 day-old chickens were divided into two dietary treatment groups using six replicate pens with 24 chickens each. No LP diet was fed in the starter phase. The protein reduction in the grower and finisher phases were 1.8 and 2% respectively. Beside the measurements of production traits, on day 24 and 40 representative fresh excreta samples were collected, their dry matter, total N, NH_4^+-N and uric acid-N contents determined, and the ratio of urinary and fecal N calculated. Dietary treatments failed to cause significant differences in the feed intake, growth rate, and feed conversion ratio of animals. LP diets decreased the total nitrogen and uric acid contents of excreta significantly. The age of birds had also significant effect, resulting more reduction in the grower phase compared with the finisher. The ratio of urinary N was higher at day 40 compared with the age of day 24. The urinary N content of broiler chicken's excreta is lower than can be found in the literature, which should be considered in the ammonia inventory calculations.

Keywords: low protein diet; broiler chickens; N excretion

Citation: Such, N.; Pál, L.; Strifler, P.; Horváth, B.; Koltay, I.A.; Rawash, M.A.; Farkas, V.; Mezőlaki, Á.; Wágner, L.; Dublecz, K. Effect of Feeding Low Protein Diets on the Production Traits and the Nitrogen Composition of Excreta of Broiler Chickens. *Agriculture* **2021**, *11*, 781. https://doi.org/10.3390/agriculture11080781

Academic Editor: István Komlósi

Received: 19 July 2021
Accepted: 12 August 2021
Published: 16 August 2021

1. Introduction

Air quality is receiving more attention worldwide. Among air pollutants ammonia is one of the most dangerous gases, contributing to biodiversity loss, general acidification of the environment and new type of deforestation [1]. Ammonia released into the atmosphere is linked to the formation of small particles with a diameter less than 10 micrometers (particular matter, PM10) [2], which can cause serious human respiratory damage (WHO, 2013). Agriculture is the main source of ammonia emission. According to the latest Hungarian air pollutant inventory report, agriculture is responsible to 92% for the total national emissions, of which about 66% takes the animal production and the ammonia emitting from the manure [3]. According to the National Emission Ceiling Directive 2016/2284, all member countries of the European Union must reduce their national emissions of air pollutants [4]. Hungary must decrease the ammonia emission by 32% until 2030, compared with 2005-year baseline. This obligation force urgent actions both on national and farm level.

Intensive poultry production using genotypes with high growth rate and egg production, resulting improved N-retention and less excretion. Dietary protein quality is a critical regulator of poultry growth, reproductive performance, and plays important role in the development of the gastrointestinal tract [5]. Protein-rich components are the most expensive ingredients in broiler diets and the member states of the European Union are dependent on the overseas soybean import [6]. A major concern for the modern poultry industry is to reduce feed cost and to optimize the protein supply of animals. Feeding low protein (LP) diets with increased crystalline amino acids could be a solution. Numerous experiments have been performed on the effect of feeding reduced protein diets on poultry growth, but the results are often contradictory [7]. Experiments in which the control and reduced protein diets had the same digestible lysine content and the diets complied with the recommendations of the "ideal protein principle" were able to achieve a 2% protein reduction without compromising the production traits [8]. Feeding LP diets has also been reported to decrease the N in excreta, thus reducing the N loss to the environment [9–11]. This is an important result, because intensive animal production systems, in particular pig and poultry farming are responsible for the emission of several air pollutants [12]. The main emitted gases are carbon monoxide, carbon dioxide and ammonia [13]. During the metabolism, the animal is utilizing only the 30–50% of the consumed nitrogen. The excreted part, mainly the urinary N is the potential source of NH_3 emission.

Several factors can influence this process, for example the species, age, live weight of animals or the protein content and amino acid composition of the diet, the housing conditions or manure management [14]. The largest proportion of nitrogen content of the chicken urine consists of uric acid and ammonia [15]. These two compounds take the total ammoniacal nitrogen (TAN), which is used in the so-called TAN-based flow models in the ammonia emission calculations [16].

The decomposition process of uric acid is carried out by urease enzyme which catalyzes the hydrolysis of urea into ammonia and carbon dioxide in aqueous medium, allowing ammonia volatilization to the atmosphere [17]. Several previous studies have shown a reduction in nitrogen excretion by about 10% with a percentage point decrease in the dietary crude protein content of broiler diets [18].

There are lots of scientific data on the ratio of excreted urinary and fecal nitrogen in mammals. However, since birds excrete feces and urine as a mixture, only few data exist on the urinary N content of poultry excreta and how this ratio change with the intensity of production, the age of birds, the dietary protein content and protein quality. The aim of this study was therefore to evaluate the effect of feeding LP diets on the performance parameters and proportion of the different nitrogenous compounds of chickens' excreta at two different age categories. The novelty of the trial is that the protein reduction of LP diets was in a range that can be applied in the practice. Furthermore, low protein diet effect on the excreta composition was studied not only at the end of the production, but also at day 24.

2. Materials and Methods

2.1. Birds and Experimental Design

The animal experiment was approved by the Institutional Ethics Committee (Animal Welfare Committee, Georgikon Campus, Hungarian University of Agriculture and Life Sciences, Deák Ferenc Street 16, 8360 Keszthely, Hungary). A total of 288 one-day-old male broilers (Ross 308) were allocated randomly to one of the 12 pens at a stocking rate of 24 birds per pen (10 bird/m^2). Each treatment was replicated 6 times. Chopped wheat straw was used as litter material.

2.2. Feed

Three phases fattening was used. The starter diet (0–10 days) was fed in mash, the grower (11–24 days) and finisher diets (25–40 days) in pelleted form. In the starter phase no LP diets were fed. The protein content of the control and LP diets in the grower phase were 21.31% and 19.49%; in the finisher phase 20.43% and 18.38%. Ad libitum feed and water were offered throughout the experiment. The composition and the measured nutrient contents of the experimental diets are shown in Tables 1 and 2. LP diets in the grower and finisher phase contained less soybean meal, but more sunflower meal and DDGS, compared with the control diets. Beside methionine, lysine, threonine, and valin, LP diets contained also crystalline isoleucine and arginine.

Table 1. Composition of experimental diets (g/kg as fed).

Ingredient	Starter	Grower		Finisher	
	Control	LP	Control	LP	Control
Corn	370	436	409	473	436
Wheat	100	100	100	100	100
Extracted soybean meal	356	113.5	243	77.2	217
Sunflower meal	50	150	100	150	100
DDGS corn	30	100	50	100	50
Sunflower oil	49.0	56.1	58.8	56.7	61.4
MCP	9.95	5.52	6.80	4.48	5.70
Limestone	18.6	17.3	16.4	16.8	15.8
L-lysine	3.71	8.48	4.06	8.27	3.42
DL-methionine	2.94	2.36	2.12	2.13	1.82
L-threonine	0.87	1.62	0.72	1.54	0.53
L-valine	0.47	1.07	0.12	0.90	-
L-isoleucine	-	1.17	-	1.32	-
L-arginine	-	0.42	-	0.59	-
Premix	5.00	4.00	4.00	5.00	5.00
Salt	2.85	2.36	2.67	2.35	2.67
Sodium bicarbonate	0.71	0.18	0.64	0.21	0.66
Phytase (Quantum blue)	0.10	0.10	0.10	0.10	0.10
Xylanase (Econase XT25)	0.10	0.10	0.10	0.10	0.10
Total	1000	1000	1000	1000	1000

Premix was supplied by UBM Ltd. (Pilisvörösvár, Pest megye, Hungary). The active ingredients contained in the premix were as follows (per kg of diet): Composition of the starter—grower premix—retinyl acetate—5.0 mg, cholecalciferol—130 µg, dl- alpha-tocopherol-acetate—91 mg, menadione—2.2 mg, thiamine—4.5 mg, riboflavin—10.5 mg, pyridoxin HCL—7.5 mg, cyanocobalamin—80 µg, niacin—41.5 mg, pantothenic acid—15 mg, folic acid—1.3 mg, biotin—150 µg, betaine—670 mg, Ronozyme® NP—150 mg, monensin-Na—110 mg (only grower), narasin—50 mg (only starter), nicarbazin—50 mg (only starter), antioxidant—25 mg, Zn (as $ZnSO_4 \cdot H_2O$)—125 mg, Cu (as $CuSO_4 \cdot 5H_2O$)—20 mg, Fe (as $FeSO_4 \cdot H_2O$)—75 mg, Mn (as MnO)—125 mg, I (as KI)—1.35 mg, Se (as Na_2SeO_3)—270 µg. Composition of the finisher premix—retinyl acetate—3.4 mg, cholecalciferol—97 µg, dl-alpha- tocopherol-acetate—45.5 mg, menadione—2.7 mg, thiamine—1.9 mg, riboflavin—5.0 mg, pyridoxin HCl—3.2 mg, cyanocobalamin—19 µg, niacin—28.5 mg, pantothenic acid—10 mg, folic acid—1.3 mg, biotin—140 µg, l-ascorbic acid—40 mg, betaine—193 mg, antioxidant—25 mg, Zn (as $ZnSO_4 \cdot H_2O$)—96 mg, Cu—9.6 mg, Fe (as $FeSO_4 \cdot H_2O$)—29 mg, Mn (as MnO)—29 mg, I (as KI)—1.2 mg, Se (as Na_2SeO_3)—350 µg.

Table 2. Measured nutrient contents of the experimental diets (%).

Ingredient	Starter	Grower		Finisher	
	Control	Control	LP	Control	LP
AMEn (MJ/kg)	12.65	13.00	13.00	13.20	13.20
Crude protein	23.95	21.31	19.49	20.43	18.38
Crude fat	7.03	8.68	8.81	8.01	8.05
Crude fiber	4.7	5.84	6.79	5.99	6.44
Ca	1.05	0.94	0,97	0.9	0.87
P	0.75	0.77	0,74	0.71	0.67
Lys	1.47	1.19	1.22	1.11	1.17
Met	0.60	0.53	0.56	0.51	0.51
Cys	0.36	0.33	0.31	0.32	0.30

Control—commercial maize-based diet; LP—Low protein diet.

2.3. Measurements

During the 40 day long fattening period, the weight gain, feed intake, and feed conversion were measured at pen basis at the end of each period (10th, 24th, and 40th day) and for the whole fattening.

On day 24 and 40 about 200 g fresh excreta samples were collected from each pen on nylon foils. Samples were mixed thoroughly, frozen, and stored at −20 °C until further processing. From these samples their dry matter, total N, NH_4^+-N, and uric acid-N contents were measured. The dry matter content of excreta samples was measured in exicator (100 °C for 24 h). The urinary N was calculated as the sum of uric acid-N and NH_4^+-N as described by O'Dell et al., (1960). Total N was determined according to the Kjeldahl method with a Foss-Kjeltec 8400 Analyzer Unit (Nils Foss Allé 1, DK-3400 Hilleroed, Denmark). The determination of NH_4^+-N content of the excreta was carried out according to the method of Peters [19]. The uric acid measurement based on the method of Marquardt et al. [20]. All N parameters were adjusted to dry matter basis.

2.4. Statistical Analyses

All data were analyzed using the Statistical Package for Social Science (SPSS) software version 22.0 (IBM Corp., Armonk, NY, USA). The performance parameters were analyzed by *t*-test, while the N contents of excreta by multivariate test of the general linear model, using the main factors of age and dietary treatments. Differences were considered significant at a level of $p \leq 0.05$.

3. Results

The effects of dietary treatments on growth performance of broiler chickens are presented in Table 3. Evaluating the dietary treatments in the grower and finisher phase, feeding LP diets slightly decreased the daily gain, feed intake, and increased the feed conversion ratio of chickens, but the differences were not significant.

Table 3. Effects of dietary treatments on the performance parameters of broiler chickens.

		Control	**LP**	**Pooled SEM**	***p*-Values**
Daily gain	Starter	237.6	227.3	3.696	0.112
	Grower	901.8	842.3	10.386	0.500
	Finisher	1593.9	1538.3	17.206	0.983
	Cumulative	2495.8	2380.7	24.168	0.756
Feed intake	Starter	314.5	301.4	3.455	0.346
	Grower	1370.2	1332.0	17.079	0.753
	Finisher	2770.0	2786.0	28.394	0.815
	Cumulative	4140.3	4118.9	42.244	0.963
Feed conversion ratio	Starter	1.32	1.33	0.030	0.757
	Grower	1.52	1.58	0.023	0.405
	Finisher	1.74	1.81	0.022	1.240
	Cumulative	1.66	1.73	0.020	1.264

Control—commercial maize-based diet; LP—low protein diet; SEM—standard error of the mean.

Similarly, to the production parameters, dry matter content of the excreta was not influenced by the treatments either (Table 4). As expected, feeding LP diets decreased the total N, NH_4^+-N and uric acid N contents of excreta. However, the decrease was significant only at day 24. Comparing with the control, the main effects of LP diets were significant decrease in all investigated N compounds. The decrease in total, uric acid, NH_4^+, fecal and urinary N were 32%, 28%, 31%, 35%, and 30% respectively. The excreta of the 40-day old birds contained significantly lower amounts of total, uric acid, fecal, and urinary N, compared with the 24-day old chickens. The decreases were in this case 23%, 18%, 29%, and 15%. The age effect on the NH_4^+-N was not significant (Table 4). In the case of the total, uric acid, fecal and urinary N significant age x dietary treatment interactions were found. Its reason was the significant age effect on these parameters in the control group, which was not the case in the LP diet treatment.

Table 4. The amount of the total-N, NH_4^+-N, uric acid-N, and dry matter content of the excreta.

Treatments		Dry Matter	Total-N	NH_4^+-N	Uric Acid N	Fecal-N	Urinary-N
		%			mg/g DM		
day 24	C	16.18	46.70 ^{a}	3.86 ^{a}	15.34 ^{a}	27.50 ^{a}	19.20 ^{a}
	LP	17.00	28.24 ^{b}	2.59 ^{b}	9.53 ^{b}	16.13 ^{b}	12.11 ^{b}
day 40	C	16.81	32.25 ^{b}	3.47 ^{ab}	11.44 ^{b}	17.35 ^{b}	14.90 ^{b}
	LP	18.10	25.50 ^{b}	2.68 ^{b}	8.99 ^{b}	13.83 ^{b}	11.67 ^{b}
				Diet effect			
Diet	C	16.49	39.48 ^{a}	3.65 ^{a}	13.39 ^{a}	22.43 ^{a}	17.05 ^{a}
	LP	17.54	26.87 ^{b}	2.63 ^{b}	9.26 ^{b}	14.49 ^{b}	11.89 ^{b}
				Age effect			
Age	day 24	16.58	37.47 ^{a}	3.23	12.43 ^{a}	21.81 ^{a}	15.66 ^{a}
	day 40	17.45	28.87 ^{b}	3.07	10.21 ^{b}	15.58 ^{b}	13.29 ^{b}
Pooled SEM		0.378	2.129	0.164	0.639	6.940	3.779
Diet		0.179	*0.000	*0.001	*0.000	*0.001	*0.000
Age		0.262	*0.005	0.561	*0.012	*0.004	*0.000
Age x Diet		0.760	*0.045	0.359	*0.048	*0.056	*0.061

Control—commercial maize-based diet; LP—Low protein diet; SEM—standard error of the mean; ^{a,b} means with different superscripts are significantly different ($p < 0.05$). *: significant at the $p < 0.05$ level

As it can be seen in Figure 1, the protein content of the diets did not modify the ratio of urinary and fecal N in the excreta. On the other hand, the excreta of the 24-day old animals contained less urinary N. This difference was significant ($p = 0.027$).

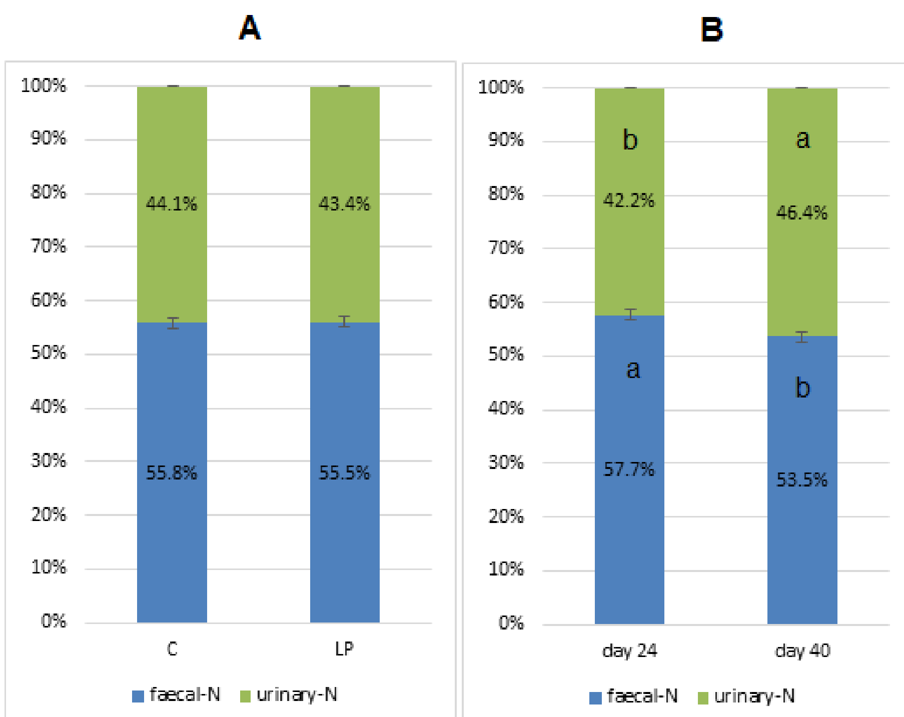

Figure 1. Effects of dietary protein content (**A**) and age of chickens (**B**) on the ratio of fecal and urinary nitrogen content of excreta. Control—commercial maize-based diet; LP—Low protein diet; SEM—standard error of the mean; a,b means with different superscripts are significantly different ($p < 0.05$).

One percent dietary protein reduction decreased the N excretion of chickens more at day 24, compared with the values at day 40 (Figure 2). The reason for this is the better feed conversion, higher N-retention of the younger animals. This means no constant factors can be used when the effects of LP diets are used for correcting the N-excretion and TAN-excretion of the animals. It should be also considered that the applied protein decrease means different relative protein reduction in the different phases—in the grower phase the relative protein reduction was 8.5%, while in the finisher period 10.0%.

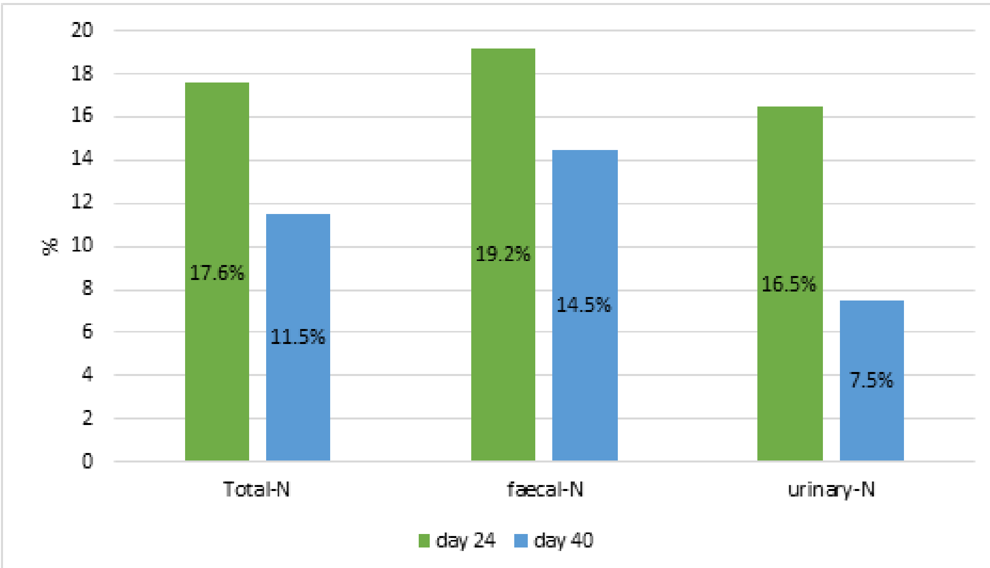

Figure 2. Effect of 1% dietary protein reduction on the decrease of excreted N of broiler chickens.

4. Discussion

Ammonia emission of poultry production is a real concern for all EU member countries and introduction of the best available practices are crucial its reduction. Nutrition has a big potential and feeding LP diets is one of the most effective options to reduce the emission. The main goal in this aspect is to find out the rate of protein reduction, which does not negatively affect the production traits and can even decrease the feeding costs [8]. Research on this often ends in unexpected results. Some experiments showed that the too high reduction cause depressive effect on performance parameters even with amino acid supplementation [21,22]. The discrepancies in responses often observed in the literature in chicks fed with low-protein amino acid-supplemented diets are related to the degree of CP reduction, the number of crystalline amino acid supplemented, the modified amino acid, the energy: protein and energy: amino acid ratios, the age of birds and the feedstuffs used [23]. Due to the changing amino acid ratios in reduced protein diets, certain amino acid deficiencies are to be expected [24]. In the case of avian species usually sulfur-containing amino acids are the first limiting, due to feather formation, but in LP diets lysine, threonine, valine, isoleucine, and arginine should also be supplemented [7]. In this experiment, the daily gain, feed intake, and feed conversion ratio of animals were the same with reduced CP diets. It means that the amino acid content of LP diets covered the requirements of the animals. These findings agree with other previous results [24], that in the case of broilers, 2% protein reduction can be achieved without impacts on the production results.

There are many factors that affect water intake of broilers, such as ambient temperature, feed intake, dietary composition, form of the feed, crude protein content, mineral metabolism, genetic factors, and the cation electrolyte balance of the feed [25–28]. According to the literature data LP diets can increase the dry matter content of the excreta and manure because of the lower water intake and urine excretion [28]. In this experiment dry matter content of excreta was not affected by the treatments, probably because of the limited, only 2% protein decrease. Similarly, in the study of van Emous et al. [29], when the protein content of the diet was 1.5% less, LP diet did not affect water intake and dry matter content of the manure, but the litter and manure samples of broilers had 8% lower total-N and 13% lower ammonia-N content.

If LP diets are used, 1% protein decrease can result about 10% decrease in the N-excretion [9]. In the study of Kerr [30], using amino acid supplemented LP diets with poultry and swine, 1% protein decrease of the diet reduced the N excretion of both species by 8.5% regardless of breed and body weight. Similar results have been published with laying hens and broilers [11,31–33]. In this study the age of chicken had significant impact on the N-excretion and on the ratio of the urinary and fecal N. In the case of 24-day old birds 1% protein decrease resulted 17.6% decrease in the total N excretion with 19.2% decrease in fecal and 16.5% in urinary N excretion. In the 40-day old chickens the decreases of the previously mentioned parameters were 11.5, 14.5, and 7.5% respectively. This means no constant values can be used in the N excretion calculations of LP diets. According to the knowledge of the authors, it is the first result that proves this age effect. It is also novel finding, that if LP diets are used, the decrease of fecal N is higher than the urinary N decrease. In the calculation of potential ammonia emission, it should also be considered.

Nitrogen-containing compounds from animal production are converted to gaseous ammonia by microbial activity [34]. Much of the ammonia released from manure comes from the hydrolysis of urea [35], or in the case of birds from the breakdown of uric acid [36].

Based on the available research results, uric acid represents 50–60% of the total N content of poultry excreta [37]. O'Dell et al. [15] found that the sum of uric acid and NH_4^+-N of the excreta gives approximately the total amount of urinary nitrogen in birds. In poultry, feces and urine are excreted together in a mixture, resulting fecal and urinary nitrogen separation difficulties. Our results prove, that the urinary N ratio of poultry excreta is less than that can be found in the literature. Using the default value of 70% TAN [16] of broiler chicken's excreta overestimates the real TAN excretion and thus the ammonia emission of poultry species. Interestingly dietary protein does not modify the fecal and urinary N ratio (55–56% vs. 43–44%). The reason for this can be, that in the case of LP diets the protein digestibility and protein utilization improves in the same manner.

Our results prove that not only the dietary factors, but also the age of birds influence the composition of the broiler's excreta. The urinary N ratio of excreta reflecting the differences in the N utilization of broiler chickens between the different age categories. The higher urinary-N content in the finisher phase means lower N-retention in this phase.

5. Conclusions

According to the results of this study 2% protein decrease of grower and finisher broiler diets did not affect the production traits but can decrease the N excretion of birds significantly. Feeding LP diets resulted into higher N-excretion reduction in younger birds. One percent protein reduction can decrease the N-excretion of 24-day old birds even by 17.6% and in the case of 40-day old animals by 11.5%. The protein content of diet does not modify the fecal: urinary N ratio of excreta, but the urinary N content of the 40-day old birds exceeds that of the 24-day old group. Due to the intensive growth rate and N-retention of broiler chickens, the urinary N ratio of excreta is lower, compared to the literature.

Author Contributions: Conceptualization, N.S. and K.D.; methodology, N.S. and L.W.; formal analysis, N.S. and K.D.; investigation, N.S., L.P., P.S., B.H., I.A.K., V.F., M.A.R. and Á.M.; data curation, L.W.; writing—original draft preparation, N.S. and K.D. writing—review and editing, N.S., V.F. and K.D.; visualization, N.S.; supervision, K.D.; project administration, V.F., K.D. and N.S.; funding acquisition, K.D. All authors have read and agreed to the published version of the manuscript.

Funding: This research was supported by the Hungarian Government and the European Union, with the co-funding of the European Regional Development Fund in the frame of Széchenyi 2020 Program GINOP-2.3.2-15-2016-00054 project.

Institutional Review Board Statement: The animal experiment was approved by the Institutional Ethics Committee (Animal Welfare Committee, Georgikon Campus, Hungarian University of Agriculture and Life Sciences) under the license number MÁB-11/2019.

Informed Consent Statement: Not applicable.

Data Availability Statement: All data generated or analyzed during this study are included in this published article.

Acknowledgments: This research was supported by the ÚNKP-20-3-II new national excellence program of the Ministry for Innovation and Technology from the source of the national research, development, and innovation fund and was prepared with the professional support of the Doctoral Student Scholarship Program of the Cooperative Doctoral Program of the Ministry for Innovation and Technology financed from the National Research, Development, and Innovation Fund.

Conflicts of Interest: All authors declare no conflict of interest.

References

1. Task Force on Reactive Nitrogen. Available online: http://www.clrtap-tfrn.org/ (accessed on 18 July 2021).
2. Giannakis, E.; Kushta, J.; Bruggeman, A.; Lelieveld, J. Costs and Benefits of Agricultural Ammonia Emission Abatement Options for Compliance with European Air Quality Regulations. *Environ. Sci. Eur.* **2019**, *31*, 1–13, doi:10.1186/S12302-019-0275-0.
3. IIR. Hungary Informative Inventory Report 1990–2019. Available online: https://www.ceip.at/status-of-reporting-and-review-results/2021-submission (accessed on 18 July 2021).
4. European Parliament. Directive (EU) 2016/2284 of the European Parliament and of the Council of 14 December 2016 on the Reduction of National Emissions of Certain Atmospheric Pollutants, Amending Directive 2003/35/EC and Repealing Directive 2001/81/EC. Brussels: European Council. *Off. J. Eur. Union* **2016**, *344*, 1–34.
5. Kamran, Z.; Aslam Mirza, M.; Mahmood, S. Effect of Decreasing Dietary Protein Levels with Optimum Amino Acids Profile on the Performance of Broilers. *Pak. Vet. J.* **2004**, *24*, 165–168.
6. Dublecz, K.; Koltay, I.A.; Such, N.; Dublecz, F.; Husvéth, F.; Wágner, L.; Péterné Farkas, E.; Márton, A.; Farkas, V.; Pál, L. Lehetőségek a Takarmányok Nyersfehérje Tartalmának Csökkentésére Monogasztrikus Állatokban. *Állatteny. Takarm.* **2018**, *67*, 273–286.
7. Belloir, P.; Lessire, M.; Berri, C.; Lambert, W.; Corrent, E.; Tesseraud, S. Revisiting Amino Acid Nutrition. In Proceedings of the 20th ESPN, Prague, Czech Republic, 24–27 August 2015; pp. 27–34.
8. Belloir, P.; Lessire, M.; Van Milgen, J.; Schmidley, P.; Corrent, E.; Tesseraud, S. Reducing Dietary Crude Protein of Broiler: A Meta-Analysis Approach. In Proceedings of the 11émes Jourmées de la Researche Avicoles et Palmipédes a Foie Gras, Tours, France, 25–26 March 2015; pp. 1–6.
9. Giner Santonja, G.; Georgitzikis, K.; Scalet, B.; Montobbio, P.; Roudier, S.; Delgado Sancho, L. *Best Available Techniques (BAT) Reference Document for the Intensive Rearing of Poultry or Pigs: Industrial Emissions Directive 2010/75/EU (Integrated Pollution Prevention and Control)*; EUR 28674 EN; European Comission: Brussels, Belgium, 2017.
10. Sterling, K.G.; Vedenov, D.V.; Pesti, G.M.; Bakalli, R.I. Economically Optimal Dietary Crude Protein and Lysine Levels for Starting Broiler Chicks. *Poult. Sci.* **2005**, *84*, 29–36, doi:10.1093/ps/84.1.29.
11. Kamran, Z.; Sarwar, M.; Nisa, M.; Mahmood, M. Effect of Low Levels of Dietary Crude Protein with Constant Metabolizable Energy on Nitrogen Excretion, Litter Composition and Blood Parameters of Broilers. *Int. J. Agric. Biol.* **2010**, *12*, 401–405.
12. De Sousa, F.C.; de Tinôco, I.F.F.; da Silva, J.N.; de Jesus Fôlgoa Baptista, F.; Souza, C.F.; Lopes Silva, A. Gas Emission in the Poultry Production. *J. Anim. Behav. Biometeorol.* **2017**, *5*, 49–55, doi:10.26667/2318-1265jabb.v5n2p49-55.
13. Angus, A.J.; Hodge, I.D.; Sutton, M.A. Ammonia Abatement Strategies in Livestock Production: A Case Study of a Poultry Installation. *Agric. Syst.* **2006**, *89*, 204–222, doi:10.1016/j.agsy.2005.09.003.
14. Battye, R.; Battye, W.; Overcash, C.; Fudge, S. *Development and Selection of Ammonia Emission Factors*; Final Report; U.S. Environmental Protection Agency: Washington, DC, USA, 1994.
15. O'dell, B.L.; Woods, W.D.; Laerdal, O.A.; Jeffay, A.M.; Savage, J.E. Distribution of the Major Nitrogenous Compounds and Amino Acids in Chicken Urine. *Poult. Sci.* **1960**, *39*, 426–432, doi:10.3382/ps.0390426.
16. EMEP/EEA. Air Pollutant Emission Inventory Guidebook 2019—Publications Office of the EU. Available online: https://op.europa.eu/en/publication-detail/-/publication/ce310211-4bc5-11ea-8aa5-01aa75ed71a1/language-en (accessed on 19 July 2021).
17. De Oliveira, M.C.; Almeida, C.V.; Andrade, D.O.; Rodrigues, S.M.M. Teor de Matéria Seca, PH e Amônia Volatilizada Da Cama de Frango Tratada Ou Não Com Diferentes Aditivos. *Rev. Bras. Zootec.* **2003**, *32*, 951–954, doi:10.1590/S1516-35982003000400022.

18. Belloir, P.; Méda, B.; Lambert, W.; Corrent, E.; Juin, H.; Lessire, M.; Tesseraud, S. Reducing the CP Content in Broiler Feeds: Impact on Animal Performance, Meat Quality and Nitrogen Utilization. *Animal* **2017**, *11*, 1881–1889doi:10.1017/S1751731117000660.
19. Peters, J.; Combs, S.; Hoskins, B.; Jarman, J.; Kovar, J.L.; Watson, M.; Wolf, A.; Wolf, N. Recommended Methods for Manure Analysis. In Proceedings of the ASA-CSSA-SSSA Annual Meeting Abstracts, Denver, CO, USA, 2–6 November 2003; ASA-CSSA-SSSA: Madison, WI, USA, 2003.
20. Marquardt, R.R.; Ward, A.T.; Campbell, L.D. A Rapid High-Performance Liquid Chromatographic Method for the Quantitation of Uric Acid in Excreta and Tissue Samples. *Poult. Sci.* **1983**, *62*, 2099–2105, doi:10.3382/ps.0622099.
21. Bregendahl, K.; Sell, J.; Zimmerman, D. Effect of Low-Protein Diets on Growth Performance and Body Composition of Broiler Chicks. *Poult. Sci.* **2002**, *81*, 1156–1167, doi:10.1093/ps/81.8.1156.
22. Namroud, N.; Shivazad, M.; Zaghari, M.; Shahneh, A.Z. Effects of Glycine and Glutamic Acid Supplementation to Low Protein Diets on Performance, Thyroid Function and Fat Deposition in Chickens. *S. Afr. J. Anim. Sci.* **2010**, *40*, 238–244.
23. Aletor, V.A.; Hamid, I.I.; Nieß, E.; Pfeffer, E. Low-Protein Amino Acid-Supplemented Diets in Broiler Chickens: Effects on Performance, Carcass Characteristics, Whole-Body Composition and Efficiencies of Nutrient Utilisation. *J. Sci. Food Agric.* **2000**, *80*, 547–554, doi:10.1002/(SICI)1097-0010(200004)80:5<547::AID-JSFA531>3.0.CO;2-C.
24. Dublecz, K.; Husvéth, F.; Wágner, L.; Márton, A.; Koltay, I.; Such, N.; Rawash, M.A.; Mezőlaki, Á.; Pál, L.; Molnár, A. Feeding Low Protein Diets Poultry and Pig Diets—Physiological, Economic and Environmental Aspects. In Proceedings of the International Symposium on Animal Science; Herceg Novi, Montenegro, 3–8 June 2019; pp. 20–29.
25. Bailey, M. The water requirements of poultry. In *Recent Developments in Poultry Nutrition 2*; Nottingham University Press: Nottingham, UK, 1999; pp. 321–335.
26. Borges, S.A.; Da Silva, A.V.F.; Maiorka, A. Acid-Base Balance in Broilers. *World's Poult. Sci. J.* **2007**, *63*, 73–81, doi:10.1017/S0043933907001286.
27. Koreleski, J.; Swiatkiewicz, S.; Arczewska-Włosek, A. The Effect of Different Dietary Potassium and Chloride Levels on Performance and Excreta Dry Matter in Broiler Chickens. *Czech J. Anim. Sci.* **2011**, *56*, 53–60, doi:10.17221/45/2010-CJAS.
28. Ziaei, N.; Guy, J.H.; Edwards, S.A.; Blanchard, P.J.; Ward, J.; Feuerstein, D. Effect of Gender on Factors Affecting Excreta Dry Matter Content of Broiler Chickens. *J. Appl. Poult. Res.* **2007**, *16*, 226–233, doi:10.1093/japr/16.2.226.
29. Van Emous, R.A.; Winkel, A.; Aarnink, A.J.A. Effects of Dietary Crude Protein Levels on Ammonia Emission, Litter and Manure Composition, N Losses, and Water Intake in Broiler Breeders. *Poult. Sci.* **2019**, *98*, 6618–6625, doi:10.3382/ps/pez508.
30. Kerr, B.J. Nutritional strategies for waste reduction management. In *Nitrogen: New Horizons in Animal Nutrition and Health*; The Institution of Nutrition of the University of North Carolina: Chapel Hil, NC, USA, 1995; pp. 47–68.
31. Lopez, G.; Leeson, S. Response of Broiler Breeders to Low-Protein Diets. *Poult. Sci.* **1995**, *74*, 685–695, doi:10.3382/ps.0740685.
32. Lopez, G.; Leeson, S. Nitrogen of Manure from Older Broiler Breeders Fed Varying Quantities of Crude Protein. *J. Appl. Poult. Res.* **1995**, *4*, 390–394.
33. Hernandez, F.; Megias, M.; Orengo, J.; Martinez, S.; López, M.J.; Madrid, J. Effect of Dietary Protein Level on Retention of Nutrients, Growth Performance, Litter Composition and NH3 Emission Using a Multi-Phase Feeding Programme in Broilers. *Span. J. Agric. Res.* **2013**, *11*, 736–746, doi:10.5424/sjar/2013113-3597.
34. Zhang, R.; Ishibashi, K.; Day, D.L. Experimental Study of Microbial Decomposition in Liquid Swine Manure, and Generation Rates of Ammonia. In Proceedings of the Livestock Waste Management Conference, American Society of Agricultural Engineers, St. Joseph, MI, USA, 16–21 June 1991.
35. Varel, V.H.; Wells, J.E.; Miller, D.N. Combination of a Urease Inhibitor and a Plant Essential Oil to Control Coliform Bacteria, Odour Production and Ammonia Loss from Cattle Waste. *J. Appl. Microbiol.* **2007**, *102*, 472–477, doi:10.1111/J.1365-2672.2006.03120.X.
36. Santoso, U.; Ohtani, S.; Tanaka, K.; Sakaida, M. Dried Bacillus Subtilis Culture Reduced Ammonia Gas Release in Poultry House. *Asian-Australas. J. Anim. Sci.* **1999**, *12*, 806–809, doi:10.5713/ajas.1999.806.
37. Nahm, K.H. Evaluation of the Nitrogen Content in Poultry Manure. *World's Poult. Sci. J.* **2003**, *59*, 77–88, doi:10.1079/WPS20030004.