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Original research paper

NUTRITIONAL STRATEGIES FOR LAYING HENS TO ADDRESS ENVIRONMENTAL CHALLENGES BY REDUCING THE NITROGEN EXCRETION

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Abstract: This research aimed to assess the impact of incorporating *Castanea sativa* powder (CSP) into laying hens diets, examining reduced crude protein (CP) levels and their effects on production performance, haematological parameters, nutrients and mineral digestibility and environmental pollution by nitrogen (N) absorption and excretion. For that, a 6-week trial was developed, with 90 Lohmann Brown laying hens aged 51 weeks, raised in digestibility cages, divided into three groups with 30 hens each. The diets were composed as follows: a control group fed with 17.50% crude protein (CON), an experimental group with a reduced CP level of 15.50% (RPL), and a similar reduced CP group supplemented with 0.5% CSP (RPC) as tannin additive. The limiting amino acids (lysine, methionine, and threonine) were supplemented to maintain constant equal amino acid concentrations in all experimental diets. Throughout the feeding trial, the laying rate was higher in the RPC group (94.12%), followed by RLP (93.65%) and CON (91.11%). However, CON hens produced heavier eggs compared to RPL and RPC groups. Average daily feed intake and feed conversion ratio showed no significant differences ($p>0.05$) between the groups. Results from blood samples showed a significant increase ($p<0.05$) in RPL group on monocytes and uric acid compared with CON and RPC groups with tendencies for leucocytes, lymphocytes, heterophiles. Notably, excreted N levels were significantly reduced (up to 30%) in RPL (0.33 mg N/100g) and RPC (0.30 mg N/100g) groups compared to the CON (0.42 mg N/100g) group, showing a promising way of reducing N pollution. The RPC group had significantly higher ($p<0.05$) N content and coefficient of apparent N absorption compared with RPL group. On the other hand, the CP excretion was significantly lower ($p<0.05$) in the RPL (2.06 mg CP/100g) and RPC (1.94 mg CP/100g) groups compared with CON group (2.63 mg CP/100g). The lowest CP absorption was determined in the RPL group, while the RPC group (88.24%), had the highest coefficient of apparent absorption, compared with both CON (86.18%) and RPL (86.22%) groups. No significant effect on mineral excretion content was observed.

Key words: *Castanea sativa, health status, poultry performances, protein levels, nitrogen balance*

INTRODUCTION

In the complex poultry farming systems, the quest for sustainable practices intertwines with the imperative of maintaining optimal animal health and productivity. Among the multitude of considerations, the management of laying hens emerges as a focal point, where the delicate ba-

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lance between nutritional requirements, environmental impact, and economic viability must be meticulously navigated (Jacobs et al., 2023). In this complex interrelationship of elements, the issue of nitrogen excretion stands as a major challenge, bearing ramifications that extend beyond the confines of the poultry house to the broader sphere of environmental sustainability.

Laying hens, prolific egg producers essential to the global food supply chain, are inherently efficient converters of feed into valuable protein sources. However, this efficiency comes hand in hand with the generation of metabolic by-products, the top among them being nitrogenous waste (Chawley, Yadav & Jagadevan, 2021). The excretion of nitrogen, primarily in the form of uric acid, poses multifaceted challenges, both within the perimeter of the poultry production system and in its wider ecological footprint (Nweze et al., 2023). From ammonia emissions with implications for air quality to nitrate leaching impacting water bodies, the ramifications of excessive nitrogen excretion reverberate across environmental domains, implicating biodiversity, human health, and ecosystem integrity (Grantz, Garner & Johnson, 2003). Against this backdrop, the imperative need to develop and implement effective nutritional strategies for laying hens takes centre stage. These strategies aim not only to optimize bird health and performance but also to mitigate the environmental footprint of poultry production, notably by reducing nitrogen excretion (Bist, Subedi, Chai & Yang, 2023). The journey towards achieving this dual objective unfolds along a continuum of scientific inquiry, technological innovation, and practical implementation, where interdisciplinary collaboration between animal nutritionists, veterinarians, agronomists, and environmental scientists becomes indispensable. Central to the attempt is the recognition that dietary interventions wield significant influence over the health and nitrogen metabolism of laying hens. From the formulation of balanced rations to the judicious selection of feed ingredients, every aspect of the hen's nutritional intake exerts a tangible impact on nitrogen utilization and excretion patterns (Vlaicu, Untea, Varzaru, Saracila & Oancea, 2023). Innovations in feed formulation, such as precision amino acid supplementation, the incorporation of alternative protein sources (Lefter et al., 2023), antioxidants and fat sources (Vlaicu, Panaite & Turcu, 2021; Liu et al., 2023) hold promise in enhancing N efficiency while sustaining egg production performance. Moreover, advancements in feed additives, ranging from enzymes that optimize nutrient digestibility (Bedford & Apajalahti, 2022) to microbial supplements that modulate gut microbiota (Yadav & Jha, 2019), offer additional avenues for fine-tuning N metabolism in laying hens (Ravindran & Abdollahi, 2017). By enhancing nutrient utilization efficiency and promoting gut health, these additives not only improve the sustainability of poultry production but also contribute to the valorization of alternative feed additives ingredients, while also improving end product quality (Lefter et al., 2023; Saracila, Untea, Varzaru, Panaite & Vlaicu, 2023; Georganas et al., 2023).

Castanea sativa, commonly known as the European chestnut, presents an intriguing avenue as a feed additive in laying hen diets with the potential to decrease N excretion (Arsenos, 2023). Rich in tannins, flavonoids, and other bioactive compounds, chestnut by-products offer multiple approaches to improving N utilization efficiency in poultry. Studies have shown that the inclusion of chestnut extracts or meals in laying hen diets can modulate N metabolism, enhancing nutrient utilization while reducing the excretion of nitrogenous waste (Buyse et al., 2021; Zaikina et al., 2022 Arsenos, 2023). This dual effect not only benefits the environment by decreasing the N load in manure but also holds promise for optimizing egg production performance and overall flock health.

This study aimed to investigate the impact of incorporating CSP into laying hens' diets, specifically exploring reduced crude protein levels, and its implications on production performance, nitrogen and protein absorption and excretion and haematological parameters.

MATERIALS AND METHODS

Animal care

The study complied with Directive 2010/63/EU and Romanian legislation (Law 206/2004, ordinance 28/31.08.2011, Law 43/11.04.2014) and was approved before the initiation of the research work by the Ethical Commission of the National Research and Development Institute for Animal Nutrition and Biology, Balotesti, Romania.

Experimental design and diets

To evaluate the effect of CSP in laying hens diets an experimental trial for 6 weeks was developed, with a total of 90 laying hens, aged 51 weeks. The laying hens were accommodated within an experimental facility and housed in Big Dutchman (Germany) three-tier cages. The environmental conditions were meticulously regulated by a Viper Touch computer system, maintaining temperature between 21.5 °C to 23 °C, humidity at 68% to 70%, and ensuring ventilation at 2% to 3%. The hens had continuous access to feed and water. Lighting followed a cycle of 16 hours of light (provided by incandescent lights at 10 lx) followed by 8 hours of darkness. The experimental facility was set to meet the sanitary-veterinary standards for the protection and welfare of laying hens used for experimental purposes. Before the feeding trial started, the hens were individually weighed, divided into 3 experimental groups with 30 hens/group, and placed in experimental cages (2 hens/cage). This cage system allowed individual cage collection of excreta for nitrogen and digestibility study, over 5 consecutive days. The hens were then randomly assigned to one of three experimental groups (CON, RPL or RPC) for the actual feeding trial. The experimental diets were formulated as follows: a control group fed a 17.50% crude protein diet (CON), an experimental group fed a 15.50% crude protein diet with reduced protein level (RPL), and a group fed the same reduced protein level diet (15.50%) and supplemented with 0.50% CSP (RPC). The limiting amino acids (lysine, methionine, and threonine) were added to each diet, respectively, to maintain constant equal amino acid concentrations in all experimental diets as presented in Table 1. Phytase was added in the two experimental groups with a lower level of protein, to provide the additional benefit of releasing nutrients, especially energy and protein. In formulating the diets for this experiment, considerations were given to the experiment's objectives, and hybrid of the hens, their age, and the nutritional guidelines provided for Lohmann Brown Classic laying hens. The chemical and mineral composition of CSP was considered in formulating the experimental diets, as presented in Table 2.

Laying hens' performance

During the 6 weeks experimental period, average daily feed intake (ADFI, g/day), feed conversion ratio (FCR, kg feed/kg eggs), laying rate (%), and egg weight (g) were monitored and recorded daily. Egg mass was calculated by multiplying the laying rate (%) by the average weight of eggs (g) divided by 100.

Table 1.
Ingredients and calculated chemical composition of experimental diets

Ingredients (%)	CON	RPL	RPC
Corn	30.11	36.35	35.85
Wheat	30.00	30.00	30.00
Soybean meal, 46% crude protein	21.00	15.22	15.22
Sunflower meal	5.00	5.00	5.00
L-lysine	-	0.16	0.16
Dl- methionine	0.10	0.16	0.16
L-threonine	-	0.01	0.01
Calcium carbonate	9.65	9.67	9.67
Monocalcium phosphate	0.73	0.78	0.78
Salt	0.37	0.37	0.37
Vegetable oil	1.98	1.22	1.22
Choline	0.05	0.05	0.05
Castanea sativa powder	-	-	0.50
Phytase	-	0.01	0.01
Premix*	1.00	1.00	1.00
Total	100.00	100.00	100.00
Calculated nutrients			
Dry matter (%)	88.78	88.72	88.72
Metabolizable energy (kcal/kg)	2720	2720	2720
Crude protein (%)	17.50	15.50	15.50
Lysine (%)	0.84	0.81	0.81
Methionine (%)	0.39	0.42	0.42
Meth + cyst (%)	0.70	0.70	0.70
Threonine (%)	0.64	0.57	0.57

*CON – control diet; RPL – experimental diet with reduced crude protein level; RPC – experimental group with reduced protein level and CSP; *The premix composition is provided elsewhere (Vlaicu et al., 2024)*

Table 2.
Chemical and mineral composition of CSP

Nutrients (%)	CSP
Dry matter	93.78
Crude protein	4.49
Crude fat	1.11
Crude fibre	5.59
Ash	2.05
Minerals (mg/kg)	
Copper	5.26
Iron	29.49
Manganese	51.26
Zinc	12.84

Determination of haematological parameters

At the end of the trial, from each of the 3 experimental groups, 6 laying hens/group were selected for blood sampling. Haematological parameters, including, haematocrit, leucocytes, heterophiles, lymphocytes, eosinophil, monocytes, creatinine, protein and uric acid, were assessed utilizing an ADVIA 2120i analyser by Siemens.

Nitrogen digestibility study

Sample collection

The apparent absorption coefficients of the nutrients were determined using the balance technique, by collecting the excreta samples during the last 5 days of the feeding trial. Excreta was collected using trays placed beneath the cages, at the same time daily, weighed, and stored individually in a refrigerator at 4 °C. After 5 days of collecting the excreta samples, average samples, consisting of six samples per group, were created, homogenized, and then oven-dried for 48 hours at 60°C. Finally, the collected excreta per cage were combined, mixed, ground, and stored for subsequent chemical analyses.

Chemical composition of CSP and excreta

The chemical composition of CSP and excreta samples was determined on samples dried at 65 °C. Standardized methods were used to determine the nutrient concentration, performed according to the Regulation (CE) no. 152/2009. Kjeldahl method was used for crude protein (Kjeltec 2300 Analyzer Unit, FOSS Analytical, Denmark). Crude fat was determined by extraction in organic solvents (Soxtec 2055—Foss Tecator, Sweden). For crude fibre the method with intermediary filtration was used (Fibertec 2010 System—Foss Tecator, Sweden). The standards used are mentioned elsewhere (Vlaicu et al., 2021).

Mineral composition of CSP and excreta

Trance mineral composition such as zinc, iron, copper, and manganese from CSP and excreta samples were determined on a Thermo Electron SOLAAR M6 Dual Zeeman Comfort (Cambridge, UK) equipment and atomic absorption spectrometry (FAAS) after microwave digestion, as described by Untea, Criste and Vladescu (2012).

Determination of nitrogen (N) and crude protein (CP) absorption, excretion and their coefficient of apparent absorption

Total N excretion was calculated by summing the nitrogen content in droppings. Similarly, crude protein excretion was determined by converting nitrogen excretion to protein using the conversion factor of 6.25. Nitrogen and crude protein absorption were calculated by subtractting the nitrogen and crude protein content in the excreta from the respective intake values.

Statistical analysis

All data were subjected to a one-way ANOVA method with Tukey's post hoc test, using Stat View for Windows (SAS, version 6.0, BrainPower Inc., 24009 Ventura Blvd. Suite 250, Calabasas, CA 91302, USA). Significant differences were set at $p<0.05$. The figures were done by GraphPad Prism, software, version 13.2 (GraphPad Software, La Jolla, CA, USA).

RESULTS AND DISCUSSION

Effect of reduced protein level and CSP on production performances

The effect of the RPL and RPC diets compared to the CON diet on the production performances of laying hens over a 6-week experimental feeding period are presented in Table 3. No significant effect ($p>0.05$) was noted for average daily feed intake and egg mass. The laying rate was significantly higher ($P<0.05$) in experimental groups compared to the CON group. The average egg weight was significantly higher ($p<0.05$) in the CON and RPL groups compared to the RPC group. A significant positive effect ($P<0.05$) was observed also for the feed conversion ratio in the RPL group than in the CON and RPC groups. These results indicate that lower protein levels in the diets of laying hens, with the addition of CSP, may improve egg production, with similar feed consumption as in a balanced diet with crude protein (17.5%). However, the CSP as a tannin supplement in the diet with reduced protein level (15.5%) did not maintain the same egg weight as the group without a tannin supplement. In the study of Nassiri Moghaddam, Kazemi Fard, Agah, Hosseini and Mirakzehi (2012), it was reported that different levels of crude protein and supplemental tallow, significantly reduced the egg weight, without detrimental effects on other egg parameters or production performances.

Although egg production can be influenced by various factors at different stages of a hen's life, our results showed that lower protein content in the RPL diet led to the production of more eggs with the same weight as those from the CON group. In contrast, the RPC group produced more eggs but with lower weight than the CON and RPL groups. Some researchers suggested that protein level, lysine and fat have varying influence on egg production and weight (Liu, Wu, Bryant & Roland, 2005; Wu, Liu, Bryant & Roland, 2005). However, there is conflicting evidence regarding the impact of protein levels on egg production. Keshavarz (2003) observed a decline in various egg production parameters when protein levels were reduced alongside the omission of supplemental methionine.

Conversely, Tenesa et al. (2016) reported that 16.5% crude protein in laying hens' diet compared to 17.5% crude protein resulted in lower feed intake, and feed conversion ratio, without affecting egg weight. More recently, Gumpha, Babu, Kumar, Samal and Panda (2019) reported that 13%, 14.5%, 16% or 17.5% of dietary crude protein levels, had no effects on egg production, egg mass, egg weight or feed conversion, but significantly decreased the body weight gain in hens with the highest crude protein level. In terms of tannins supplement, Cornescu et al. (2022) reported a significant increase in production performances, when 0.5% chestnut powder in contrast to 0.5% oak bark and control, however, the authors used a similar protein level (17.5%) in laying hens' diet.

Nevertheless, although tannins have traditionally been considered anti-nutritional factors in monogastric nutrition with negative effects on feed intake, nutrient digestibility and production performance, several studies have shown that when used in low supplemental concentrations they can improve animals' health, and production performances and nutrition (Brus, Dolinšek, Cencič & Škorjanc, 2013; Starčević et al., 2015; Huang, Liu, Zhao, Hu & Wang, 2018).

Effect of reduced protein level and CSP on haematological paramters

The results regarding the effect of different dietary levels of crude protein levels and CSP on the haematological parameters of laying hens are presented in Table 4. The inclusion of CSP, as a tannin additive in the diet led to a significant decrease in monocytes compared to the RPL group. Specifically, monocytes decreased by 48.58% in the group receiving CSP compared to the RPL group, and by 32.93% compared to the CON group. Similarly, the addition of CSP resulted in a significant decrease in uric acid levels compared to the RPL group, with a reduction of 30.12%. Additionally, there was a 9.41% decrease compared to the CON group, which, however, did not reach significance ($p>0.05$).

While lower levels of haematocrits, leucocytes, and heterophiles were observed in the groups with reduced protein levels, these differences were not statistically significant ($p>0.05$) compared to the CON group. This suggests that tannin supplementation may have a beneficial effect on monocyte levels, potentially indicating an improvement in the hens' immune response or inflammatory status. Lower levels of uric acid can be indicative of improved kidney function or reduced protein catabolism. This finding suggests that tannin supplementation might positively impact uric acid metabolism in laying hens. Similarly, Gumpha et al. (2019) reported that uric acid decreased from 6.02 mg/dL to 3.38 mg/dL, being directly correlated with the crude protein reduction from 17.5% to 13% in laying hens. The same authors reported significant alterations in the serum of laying hens fed with reduced crude protein levels including the enzyme activity from blood serum. However, literature data is scarce in reporting the results regarding the simultaneous reduction of crude protein and tannin supplements in laying hens. Generally, in monogastric nutrition, tannins have been studied in pigs for several reasons, as reviewed by Huang et al. (2018). Later, Choi and Kim (2020) reviewed the effects of tannins on broiler chickens and laying hens, concluding that tannins can elicit either beneficial or detrimental effects on the growth performance and gut ecosystem of the broiler chickens. They have also mentioned that supplementation of tannins alone or in conjunction with other strategies has large potential to alleviate challenges, replace antibiotic growth promoters and im-prove production efficiency in poultry pro-ductions. Similarly, Kumar et al. (2022) also noted that while tannins hold promise as feed additives, their efficacy varies depending on factors such as source, chemical composition, age, and poultry species. Despite their potential to mitigate various issues, substitute antibiotic growth promoters, and enhance overall poultry health and productivity, the optimal inclusion level of tannins in feed formulations remains subject to these variables. Overall, the inclusion of 0.50% CSP into laying hens' diets with reduced protein levels has shown promising effects on both haematological parameters and general health status.

Specifically, supplementation has been associated with favourable changes in haematological markers such as decreased levels of monocytes and uric acid, indicating potential improvements in immune function and metabolic health.

Effect of reduced protein level and CSP on chemical and mineral composition of excreta samples

The impact of dietary interventions on the digestibility of proximate composition is detailed in Table 5. Findings indicate that the levels of nutrients such as crude fat, crude fibre, and ash did not show significant differences (p>0.05) among the dietary groups, indicating that altering the protein content of the diet did not impact these nutrient levels significantly. However, nitrogen levels exhibited a significant reduction (p<0.05) in the RPL and RPC group compared to the CON group. When considering mineral content, there were no significant differences (p>0.05) observed among the groups.

This suggests that neither the reduced protein level nor the specific additive has a substantial effect on copper content in the diet. While not statistically significant (p = 0.0763), there was a noticeable trend towards increased iron levels in both the RPL and RPC groups compared to the CON group. This suggests that reducing protein levels in the diet might have a slight influence on iron content, potentially due to alterations in feed composition or absorption dynamics. Similar to iron, there was a trend towards higher manganese levels in the RPL and RPC groups compared to the CON group, although this difference was not statistically significant (p =0.1113).

Table 3.
Effect of RPL and RPC compared to CON diet on production performance of laying hens.

Specification	CON	RPL	RPC	SEM	p
Average daily feed intake (g/day)	108.09	106.41	106.60	0.323	0.0659
Laying intensity (%)	91.11 ^{b}	93.65 ^{a}	94.12 ^{a}	0.408	0.0044
Average egg weight (g)	65.09 ^{a}	64.76 ^{a}	62.72 ^{b}	0.242	<0.0001
Feed conversion ratio (kg feed/kg egg)	1.84 ^{a}	1.76 ^{b}	1.82 ^{a}	0.011	0.0101
Egg mass (g)	59.30	60.01	59.65	0.337	0.6979

CON – control diet; RPL – experimental diet with reduced crude protein level; RPC – experimental group with reduced protein level and CSP; ^{a,b}Different superscript letters within a same row denote significant differences between the means at p<0.05

Table 4.
Effect of RPL and RPC compared to CON diet on haematological parameters of laying hens

Specification	CON	RPL	RPC	SEM	p
Haematocrits (%)	23.83	22.00	21.00	0.656	0.2092
Leucocytes (K/uL)	15.13	13.97	13.07	0.747	0.5554
Heterophiles (%)	38.83	37.33	35.40	1.996	0.8098
Lymphocytes (%)	54.20	51.20	58.25	1.390	0.1395
Monocytes (%)	9.20 ^{ab}	12.00 ^{a}	6.17 ^{b}	0.949	0.0305
Eosinophils (%)	3.75	5.17	4.50	0.524	0.6083
Creatinine (mg/dL)	0.06	0.06	0.06	0.002	0.3548
Serum proteins (g/dL)	5.37	5.38	5.27	0.099	0.8876
Uric acid (mg/dL)	3.85 ^{b}	4.25 ^{a}	2.97 ^{b}	0.213	0.0326

CON – control diet; RPL – experimental diet with reduced crude protein level; RPC – experimental group with reduced protein level and CSP; ^{a,b}Different superscript letters within a same row denote significant differences between the means at p<0.05

Table 5.
Effect of RPL and RPC compared to the CON diet on chemical and mineral composition of laying hens' excreta

Specification	CON	RPL	RPC	SEM	p
Proximate composition (mg/100g)					
Dry matter	23.79	23.70	22.89	0.055	0.5621
Nitrogen	1.47[a]	1.25[b]	1.19[b]	0.035	0.0007
Crude fat	0.55	0.60	0.58	0.529	0.4749
Crude fibre	3.44	3.54	3.29	0.061	0.2436
Ash	5.76	5.82	5.38	0.132	0.3547
Minerals (mg/100g)					
Copper	8.84	8.65	8.88	0.309	0.9497
Iron	350.55	390.40	387.08	6.713	0.0763
Manganese	97.37	109.86	105.92	2.499	0.1113
Zinc	89.17	103.81	98.99	3.946	0.3141

CON – control diet; RPL – experimental diet with reduced crude protein level; RPC – experimental group with reduced protein level and CSP; [a,b]Different superscript letters within a same row denote significant differences between the means at p<0.05

This again suggests that dietary changes could impact manganese content, but further investigation may be needed to confirm this trend. Zinc levels show a similar trend to iron and manganese, with higher values in both the RPL and RPC groups compared to the CON, but not statistically significant (p=0.3141), suggesting a potential influence of dietary modifications on zinc content, although the exact mechanisms behind this trend require further exploration. However, since no significant effect was noted for trance minerals this suggests that the variations in dietary protein levels did not influence the absorption or excretion of these minerals.

However, the decrease in dietary crude protein levels in both the RPL and RPC groups likely contributed to reduced levels of crude protein in the excreta. This phenomenon mirrors findings reported by Heo et al. (2023) when feeding laying hens with reduced protein levels. Animals utilize dietary protein for various physiological functions, such as growth and enzyme production. Consequently, when dietary protein intake is diminished, less protein is available for these functions, resulting in lower levels of crude protein being excreted in the droppings of laying hens, as noted by Desbruslais, Wealleans, Gonzales-Sanchez and di Benedetto (2021). The significant decrease in nitrogen levels observed in the excreta of these groups supports this conclusion. Since protein serves as a primary source of nitrogen in the diet, a reduction in dietary protein content would naturally lead to decreased nitrogen intake and subsequent excretion, as previously observed (Latshaw & Zhao, 2011). These insights highlight the potential of tailored dietary intervenetions to not only improve nutrient utilization efficiency but also mitigate nitrogen emissions, contributing to more sustainable poultry production practices and reduced environmental impact.

Effect of reduced protein level and CSP on N and CP absorption and excretion

The impact of reduced levels of crude protein with or without CSP on N and CP absorption and excretion is presented in Fig. 1. Notably, the RPL group exhibited significantly lower (p<0.05) Nabsorption (1.98 mg N/100g) compared to the CON group (2.67 mg N/100g) but without significant effect compared to the RPC group (2.36 mg N/100g) (Fig. 1A).

This reduction in N absorption corresponded with significantly lower (p<0.05) nitrogen excretion observed in both groups with reduced protein levels (0.33 mg N/100g in RPL group and 0.30 mg N/100g in RPC group) relative to the CON group (0.42 mg N/100g) (Fig. 1B). The coefficient of N apparent absorption showed significant (p<0.05) effect only between the RPL (85.50%) compared to RPC (88.04%) groups, while none of them being different compared to CON group (86.64%) (Fig.1C). A similar trend was observed for CP absorption, excretion and their coefficient of CP apparent absorption in these groups. Specifically, the CP absorption was significantly (p<0.05) lower in the RPL group (13.56 mg CP/100g) compared. These findings harmonise with the results reported by Heo et al. (2023). The significance of these results lies in their implications for sustainable poultry production Reducing N excretion is very important for environmental sustainability, and the reduction of dietary protein

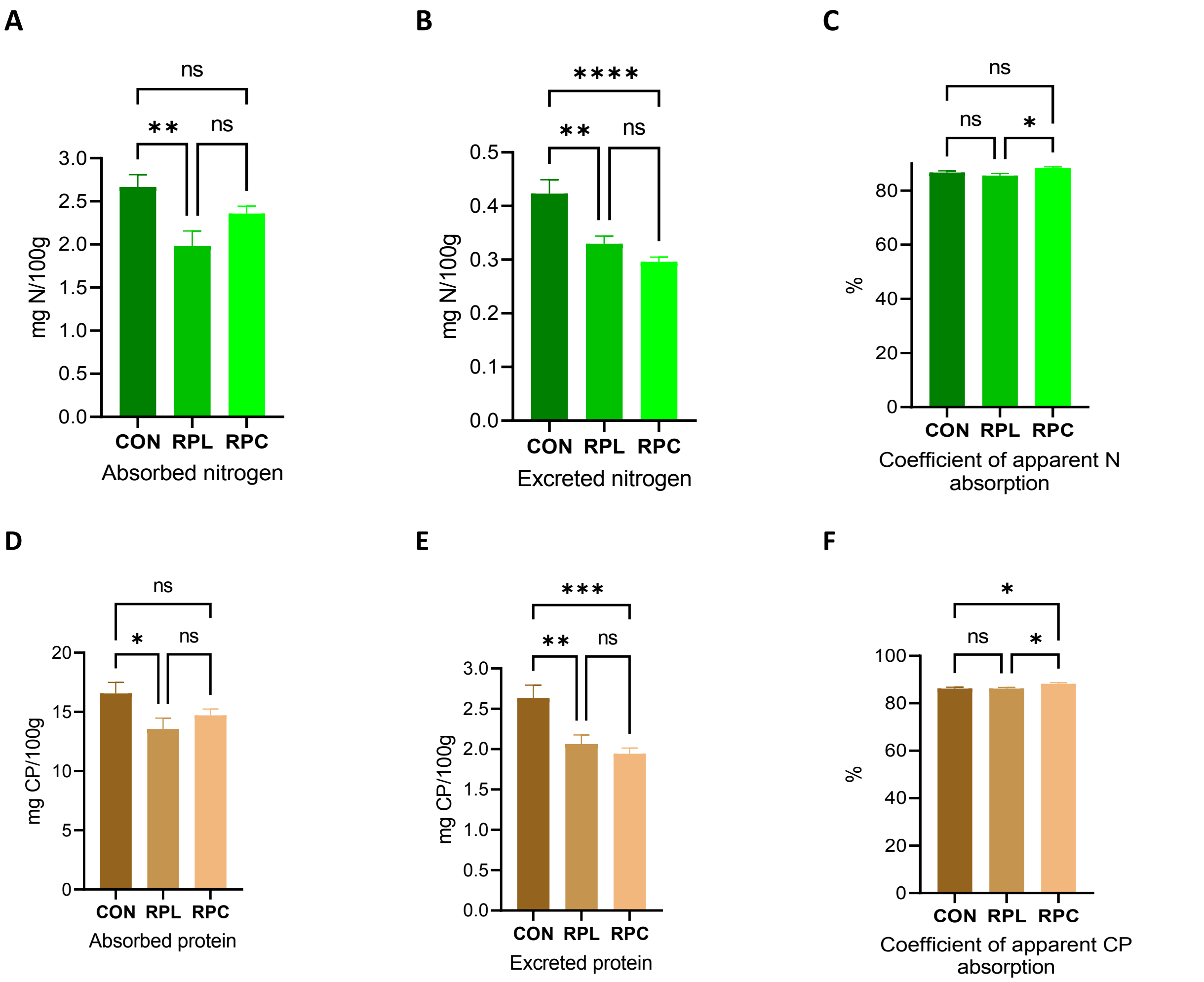

Figure 1. Effect of RPL and RPC diets compared to CON diet on nitrogen and protein absorption and excretion. CON – control diet; RPL – experimental diet with reduced crude protein level; RPC – experimental group with reduced protein level and CSP; * p<0.05, **<0.001,***p<0.0001, ns – non significant

in laying hens' diets emerges as a prominent strategy in this regard.only with the CON group (16.57 mg CP/100g), with no effect for the RPC group (14.71 mg CP/100g) (Fig. 1D). However, the highest ($p<0.05$) CP excretion was observed in the CON group (2.63 mg CP/100g), compared with both RPL (2.06 mg CP/100g) and RPC (1.94 mg CP/100g) groups (Fig. 1E). In contrast, the higher apparent coefficient of CP absorption was determined in the RCP group (88.24%), compared with both CON (86.18%) and RPL (86.22%) groups (Fig. 1D).

The observed decrease in nitrogen absorption and subsequent excretion in response to reduced dietary protein levels can be attributed to several factors. Lowering dietary protein levels may enhance nitrogen utilization efficiency in the birds' metabolism as reported in the meta-analysis of Alfonso-Avila, Cirot, Lambert and Létourneau-Montminy (2022). With reduced CP intake, there was less excess N available for excretion, resulting in de-creased N excretion. Additionally, dietary CP levels influence protein metabolism processes within the body. Reductions in dietary CP may lead to adjustments in protein metabolism path-ways, resulting in decreased N excretion, which explains the current findings.

Moreover, tannin supplementation may further enhance N retention and utilization efficiency. Tannins have been reported to modulate protein metabolism and improve nitrogen retention in poultry, contributing to the observed reduction in nitrogen excretion (Buyse et al., 2021; Darmawan et al., 2022). Overall, the findings underscore the efficacy of reducing dietary CP levels, supplemented with tannins if applicable, as a viable strategy for mitigating N excretion in laying hens, thereby promoting sustainable poultry production practices.

Introducing CSP into laying hens' diets with reduced CP levels represents a promising approach aimed at reducing N excretion, as the tannins present in CSP are known to bind with proteins, leading to decreased N excretion and soil pollution. Additionally, the supplementation may enhance CP absorption efficiency, as tannins are beneficial to gut health and nutrient utilization, thereby potentially optimizing protein uptake by the hens' digestive system.

CONCLUSIONS

Reducing CP levels in laying hens' diets increased egg production, but might decrease egg weight, without affecting feed intake and feed conversion ratio. Laying hens' haematological parameters were not affected by reduced protein content or CSP addition. Supplementing CP content reduced diets with tannins from CSP as feed additives resulted in a significant reduction in N excreta while significantly increasing N and CP absorption. The tested diets had no significant effect on minerals in the excreta samples. Finally, CSP in laying hens' diets with reduced CP levels demonstrates potential as an effective approach, offering benefits such as reduced N excretion and potentially improved CP absorption efficiency, thereby contributing to optimized performances and reduced environmental impact.

AUTHOR CONTRIBUTIONS

Conceptualization, P.A.V.; Methodology, P.A.V., A.E.U., T.D.P., M.G.C., M.C., I.V., and A.G.O.; Investigation, P.A.V.; formal analysis, P.A.V., A.E.U., T.D.P., M.G.C., M.C., I.V., and A.G.O.; validation, A.E.U.; writing-original draft preparation, P.A.V.; Writing-review and editing, P.A.V. and A.E.U.

DATA AVAILABILITY STATEMENT

Data contained within the article.

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CONFLICT OF INTEREST

The authors declare no conflict of interest. The funders had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript, or in the decision to publish the results.

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NUTRITIVNE STRATEGIJE U ISHRANI KOKA NOSILJA U SVETLU REŠAVANJA IZAZOVA U ZAŠTITI ŽIVOTNE SREDINE SMANJIVANJEM IZLUČIVANJA AZOTA

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Sažetak: Ovo istraživanje je imalo za cilj da proceni uticaj uključivanja praha pitomog kestena *Castanea sativa* (CSP) u ishranu koka nosilja, ispitujući smanjene nivoe sirovih proteina (CP) i njihove efekte na performanse proizvodnje, hematološke parametre, hranljive materije i mineralnu svarljivost i zagađenje životne sredine apsorpcijom i izlučivanjem azota (N). Eksperimentalni dizajn se sastojao od 6-nedeljnog ogleda sa 90 Lohmann Braun koka nosilja starosti 51 nedelja, odgajanih u kavezima za probavljivost, podeljenih u tri grupe sa po 30 koka. Režim ishrane je bio sledeći: kontrolna grupa je hranjena sa 17,5% sirovih proteina (CON), jedna eksperimentalna grupa (RPL) sa smanjenim udelom proteina (15,5% CP) i slična grupa (RPC) sa redukovanim proteinima dodatno suplementirana sa 0,5% CSP kao izvorom tanina. Limitirajuće amino kiseline (lizin, metionin i treonin) su dodavane, tako da njihovi nivoi budu isti kod svih eksperimentalnih grupa. Tokom eksperimenta, stopa polaganja jaja je bila veća u RPC grupi (94,12%), a zatim u RLP grupi (93,65%) i 91,11% u kontrolnoj grupi. Nije pokazana statistički značajna razlika ($p<0,05$) između eksperimentalnih grupa u prosečnom unosu i konverziji hraniva. Rezultati krvnih analiza su pokazali značajno povećanje ($p<0,05$) sadžaja monocita i urinske kiseline u RPL grupi u poređenju sa CON i RPC sa tendecijama za leukocite, limfocite i heterofile. Količine izlučenog azota su značajno smanjene ($p<0,05$) (do 30%) u RPL (0,33 mg N/100g) i RPC grupi (0.30 mg N/100g) u poređenju sa kontrolnom (CON) grupom (0,42 mg N/100g), što ukazuje na potencijal za redukovanje zagađenja okoline azotom. Grupa RPC imala je značajno ($p<0,05$) veći sadržaj azota i koeficijent prividne apsorpcije azota u poređenju sa RPC grupom. S druge strane, ekskrecija CP je bila značajno ($p<0,05$) niža u RPL (2,06 mg CP/100g) i RPC (1,94 mg CP/100g) grupama u poređenju sa kontrolnom grupom (2,63 mg CP/100g). Najniža CP apsorpcija je izmerena u RPL grupi, dok je RPC grupa imala najveći koeficijent prividne apsorpcije (88,24%) u poređenju sa kontrolnom (86,18%) i RPL (86,22%) grupom. Nije zabeležen značajni efekat na izlučivanje minerala.

Ključne reči: *Castanea sativa, zdravstveni status, performanse živine, nivoi proteina, balans azota*

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