Scientific Papers. Series D. Animal Science. Vol. LXVII, No. 2, 2024
ISSN 2285-5750; ISSN CD-ROM 2285-5769; ISSN Online 2393-2260; ISSN-L 2285-5750

EFFECTS OF DIETARY OAK BARN ON PERFORMANCE TRAITS AND NITROGEN BALANCE IN LAYING HENS

Iulia VARZARU, Arabela Elena UNTEA, Tatiana PANAITE, Gabriela Maria CORNESCU, Mihaela SARACILA, Alexandra Gabriela OANCEA, Petru Alexandru VLAICU

National Research and Development Institute for Biology and Animal Nutrition - IBNA Balotesti, Calea Bucuresti, No.1, 077015, Balotesti, Ilfov, Romania

Corresponding author email: iulia.maros@ibna.ro

Abstract

This study aimed to evaluate the effectiveness of oak barn as a natural source of tannins included in low protein diets on the performance parameters, excreta composition, and nitrogen balance of laying hens. A total of 168 Lohmann Brown laying hens, 51 weeks of age, were divided into three dietary treatment groups and housed in digestibility cages. Laying hens were fed 17.5% crude protein (CP) in control 1 (C1), 15.5% CP in control 2 (C2), and 15.5% CP supplemented with 0.5 % oak barn in the experimental group (E). Dietary treatments registered a significant increase (p = 0.001) in laying production in the C2 and E groups, and a significant decrease in average egg weight (p = 0.0002) in the E group. The nitrogen balance assessment showed a significant decrease (p = 0.0004) of the nitrogen excreta in groups with reduced dietary protein (C2 and E) compared to control (C1). The cumulative effect of the two studied factors (oak bark and protein level) led to a reduction in nitrogen elimination through droppings of almost 33%, on average.

Key words*: nitrogen excreta, pollution, poultry, reduced dietary protein, tannins.*

INTRODUCTION

Creating a sustainable and competitive egg-production system presents a significant challenge for the laying-hen industry (Gautron et al., 2021). Although poultry production isn't a primary source of harmful gases, the excretion of nitrogen in intensive poultry production is responsible for concerning environmental issues (Gržinić et al., 2023). Additional focus is required to develop nutritional solutions for reducing nitrogen excretion and mitigating the emissions of noxious and greenhouse gases (Malomo et al., 2018). The protein content in feed shows a strong correlation with nitrogen emissions in excreta (Heo et al., 2023). Consequently, low-protein diets are widely recognized as a nutritional strategy to curb ammonia emissions while cutting down feed expenses by diminishing the need for high-cost protein sources in the overall diet (Such et al., 2021). Moreover, enhancing the digestibility of dietary protein proves to be an effective strategy for reducing nitrogen excretion (Dukhta & Halas, 2023).

Tannins, defined as a heterogeneous group of polyphenolic compounds, are secondary metabolites produced by plants as a nonspecific defense mechanism (Choi & Kim, 2020). Tannins are found in different parts of plants, like seeds, bark, wood leaves, and fruit skins, and exhibit a wide range of biological properties, such as antimicrobial, anti-parasitic, antiviral, antioxidant, anti-inflammatory, and immunomodulatory effects (Huang et al., 2018). They are primarily categorized into three main groups: hydrolyzable tannins, condensed tannins, and phlorotannins (Englmaierová et al., 2022). The condensed tannins have a higher molecular weight and more complex structure compared to hydrolyzable tannins, which could suggest a reduced bioavailability within the gastrointestinal tract of poultry (Serrano et al., 2009). Tannins have been considered antinutrients due to their association with various negative effects, such as decreased feed conversion reduced bioavailability of micronutrients, and reduced growth, these effects being particularly attributed to condensed tannins (Selle et al., 2010). Some authors suggested that a low concentration of tannins can improve feed palatability and increase the performance of monogastrics by stimulating consumption (Windisch & Kroismayr, 2006). Other authors reported that stimulation of digestive secretions may be the main mechanism of action for tannins (Li et al., 2020).

Quercus robur L., known as the common oak, is a well-known species of tree, spontaneously found in deciduous forests and utilized both in the wood industry and traditional folk medicine practices for the treatment of wounds and skin diseases (Dróżdż & Pyrzynska, 2018). In the wood industry, the bark is often deemed as waste material (Elansary et al., 2019). Oak bark comprises various polyphenols, including vanillic acid, ellagic acid, and gallic acid (De Simon et al., 1996).

Oak bark extract has been used as an anti-inflammatory agent, due to the elevated content of tannins, pectins, and flavonoids, which exhibit antimicrobial properties. Oak bark supplementation into broilers' diets resulted in increased feed intake without adverse effects on body weight (Bagno et al., 2018). Furthermore, supplementation of oak bark extracts along with enzyme preparations enhanced digestion. The inclusion of *Quercus* cortex extract, which contains biologically active compounds, into the ration of broiler chickens enhances productivity and strengthens the immune-modulating body state (Fisinin et al., 2018). Duskaev et al. (2021) showed that the addition of oak bark extract in diets can stimulate the accumulation of essential and nonessential amino acids mainly in the pectoral muscles of broilers, having a great economic value. Moreover, it was suggested that oak bark as a phytogenic feed additive can increase feed digestibility, stimulate growth, improve feed conversion ratio, and increase feed intake in broilers. Bagirov et al. (2018) reported that dietary *Quercus* cortex extract can enhance the slaughter indices of broilers, modify the fatty acid profile and the mineral composition of muscle, and impact the microbiome of the small intestine.

The goal of this study was to assess the effect of the oak barn as a natural source of tannins included in low protein diets on the performance parameters, excreta composition, and nitrogen balance of laying hens.

MATERIALS AND METHODS

The experimental procedures were conducted with approval from the Ethics Committee of the National Research and Development Institute of Animal Biology and Nutrition, Romania. These procedures adhered to the guidelines outlined in Romanian Law 43/2014 for the handling and protection of animals utilized for experimental purposes, as well as Directive 2010/63/EU concerning the protection of animals used for scientific purposes.

Experimental design

A six weeks experiment was conducted on 168 Lohmann Brown laying hens, 51 weeks old, which were assigned into 3 groups and housed in metabolic cages (Zucammi batteries Z.M.E.C 50-model 2012) that allow accurate weighing of the daily ingesta and excreta, and controlled environmental conditions. During the experimental period laying hens received the following experimental diets: a control diet (C1) containing a corn–soybean diet, a control diet with low-protein content (C2), and an experimental diet (E) with the same structure as the C2 diet and with an additional 0.5% oak bark supplement (Table 1). The oak bark used in the experiment was purchased from a local producer, and then dried, grounded, and packed. Feed and water were supplied *ad libitum*.

During the experimental period, daily records were kept for feed intake, feed conversion ratio, egg production, egg weight, and laying percentage.

During the last 5 days of the experiment, excreta samples were daily collected. Droppings samples were homogenized, dried in the drying oven BMT model Ecocell Blueline Comfort (Nuremberg, Germany) for 48 h, at 65°C and ground with Grindomix GM 200 mill (Retsch, Germany).

Chemical analyses

Feed samples were analyzed to assess the proximate composition as follows: dry matter (method 930.15; AOAC, 2005), crude protein with Tecator Kjeltek auto 1030 analyzer (SR

EN ISO 5983-2, 2009), ether extract with continuous extraction in a solvent, followed by fat measurement with Soxhlet after solvent removal (SR ISO 6492, 2001), and ash (method 942.05; AOAC, 2007). Excreta samples were analyzed for nitrogen assessment using the same methodology as for feed samples.

Nitrogen balance was assessed using the analytical data generated by the chemical analysis of the feeds and droppings, and the recorded data regarding daily feed intake and excreta. The coefficients of protein digestibility were determined using digestibility equations proposed by Sirirat et al. (2013).

Table 1. Diets formulation

Ingredients	C1	C2	E
Corn, %	30.11	36.35	35.85
Wheat, %	30	30	30
Soybean meal, %	21	15.22	15.22
Sunflower, %	5	5	5
Dl- methionine, %	0.1	0.16	0.16
L-lysine	-	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
Premix, %	1	1	1
Bark oak, %	-	-	0.5
Phytase, %	-	0.01	0.01
Total	100	100	100
Chemical analysis (calculated values)			
Metabolizable energy, (kcal/kg)	2720	2720	2720
Dry matter, %	88.78	88.72	88.72
Crude protein, %	17.5	15.5	15.5
Lysine, %	0.84	0.81	0.81
Methionine, %	0.39	0.42	0.42
Met+cist, %	0.7	0.7	0.7
Treonina, %	0.64	0.57	0.57

Statistical Analysis

The analytical data were subjected to statistical analysis through a two-way ANOVA, followed by Tukey's test, using XLSTAT software (Addinsoft, France) and Prism-GraphPad software v. 9.1.2 (San Diego, CA, USA). A probability level below 5% was considered significant.

RESULTS AND DISCUSSIONS

The effects of dietary treatments on the productive performances of laying hens are presented in Figure 1. Feeding low protein diets slightly decreased feed conversion ratio and average egg weight but the differences were not significant. Supplementation with oak bark of low protein diets led to a significant ($p < 0.001$) reduction of daily feed consumption and average egg weight, compared to the control group, while feed conversion ratio and laying intensity were not influenced by the dietary treatments.

Results of this study showed that average egg weight significantly decreased in the groups with reduced dietary protein. Similar to our results, Heo et al. (2023) reported a significant decreasing effect of weights of eggs as the reducing content of crude protein from diets, although there was a linear decrease ($P < 0.05$) in nitrogen excretion observed as dietary crude protein levels decreased, both in pullets and laying hens. This finding aligns with previous research indicating that reducing crude protein levels in diets results in decreased egg weight (Alagawany et al., 2020; Summers & Leeson, 1994). Ji et al. (2014) also noted that reduced protein concentrations did not impact growth performance and egg production, being expected that decreasing crude protein levels in chicken diets would serve as an efficient dietary strategy to mitigate the environmental impact of nutrient overload.

The chemical composition of the excreta samples was analyzed and presented in Table 2.

Table 2. Chemical analysis of laying hens' excreta

Items	C1	C2	E	SEM	P value
DM, %	25.58 ^{a}	24.44 ^{a}	23.42 ^{a}	0.532	0.262
CP, %	9.19 ^{a}	8.09 ^{a}	8.29 ^{a}	0.208	0.059
EE, %	0.52 ^{a}	0.73 ^{b}	0.61 ^{ab}	0.028	0.005
CF, %	3.44 ^{a}	3.54 ^{a}	3.35 ^{a}	0.065	0.503
CEN, %	5.76 ^{a}	5.82 ^{a}	5.59 ^{a}	0.239	0.504

Values with different superscripts in the same row are significantly different ($P < 0.05$).

Similar to the effects on production parameters, the dry matter content of the excreta was not influenced by the dietary treatments. As expected, feeding low protein diets led to a reduction in the crude protein concentrations in excreta samples from C2 and E groups, compared to the C1 group, but the differences were not statistically significant. Contrary, crude fat concentrations from excreta samples increased significantly ($p < 0.05$) in the group with reduced dietary protein levels without oak bark supplementation (C2).

Adding a source of tannins (oak bark) in the reduced protein diet (E) also led to a slight increase in crude fat concentrations from excreta, but not significantly different, compared to the control group (C1).

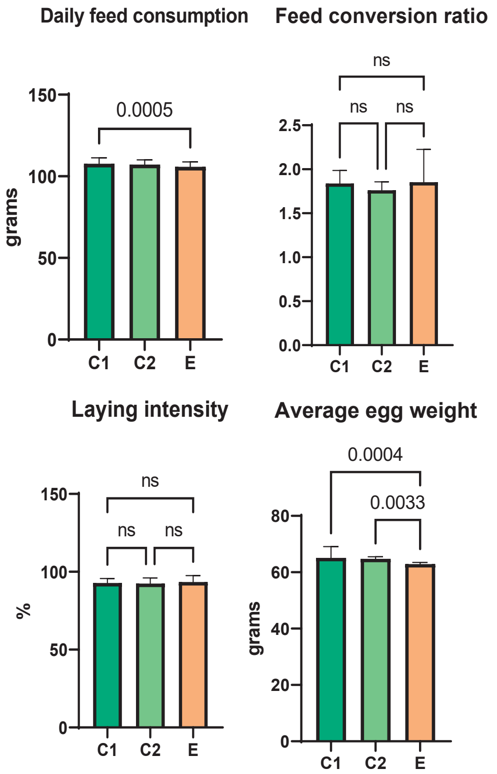

Figure 1. The influence of dietary oak bark supplementation and protein level on productive performances

The excreta collected during the balance period contained significantly ($p < 0.05$) lower amounts of nitrogen in groups with low protein diets (C2 and E), compared to the control group (Table 3). The concentrations of nitrogen decreased by 21.7 % in the group with reduced dietary content of crude protein (C2) and by 32.6 % in the group with low protein diet and dietary oak bark supplementation (E), compared to the control group (C1) (Figure 2). Ammonia emissions from poultry production represent a significant concern, necessitating the implementation of the best available practices to mitigate them. Nutrition plays a pivotal role, with feeding low-protein diets emerging as one of the most effective strategies for reducing emissions. The primary objective is to determine the optimal protein reduction level that maintains production traits without adverse effects and potentially reduces feeding costs (Belloir et al., 2015).

When employing low-protein diets, a reduction of 1% in protein content can lead to approximately a 10% decrease in nitrogen excretion (Santonja et al., 2017). Nitrogen found in droppings of laying hens originates from a mixture of undigested dietary crude protein, basal endogenous losses, and microbial fermentation by-products (Soomro et al., 2018). It is documented that excreted nitrogen can contribute to issues like eutrophication, nitrous oxide emissions, and global warming (Aneja et al., 2006). Hence, decreasing nitrogen elimination is crucial for fostering sustainable production. Implementing reduced protein diets remains a significant approach for minimizing nitrogen elimination in droppings. In a study conducted by Kerr et al. (1995), which involved the use of diets with reduced protein content and with supplementation of amino acids for poultry, a 1% decrease in dietary protein resulted in an 8.5% reduction in nitrogen excretion, irrespective of breed or body weight.

In this study, a reduction of crude protein by 2 % from laying hens' diets was applied, resulting in a 21.7 % decrease in the nitrogen excreta (C2 group) compared to the control group. The results of this research are in line with previous studies reporting that nitrogen excretion decreased linearly as the dietary crude protein levels decreased (Soares et al., 2019; Alfonso-Avila et al., 2022). The reduction in nitrogen excretion associated with decreased crude protein concentrations in diets can be associated with reduced nitrogen intake. Such et al. (2021) showed that a reduction of 2% in protein content in grower and finisher broiler diets had no adverse effects on production traits, but significantly decreased the nitrogen excretion of the birds. The same authors reported that feeding low-protein diets led to greater reductions in nitrogen excretion in younger birds. A 1% reduction in protein content could decrease nitrogen excretion by as much as 17.6% in 24-day-old birds and by 11.5% in 40-day-old animals.

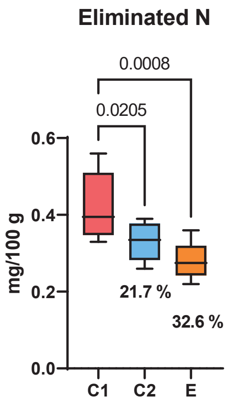

Figure 2. The influence of dietary oak bark supplementation and protein level on nitrogen excretion

Protein digestibility and nitrogen balance were assessed based on the values of feed consumption and eliminated amounts, along with the chemical composition of feeds and droppings. Protein digestibility tended to decrease in the group with reduced protein (C2) and increased in the group with supplemented oak bark in the reduced protein diet (E), compared to control. However, statistical differences were not observed in protein digestibility between the groups.

Table 3. The influence of dietary oak bark supplementation and protein level on crude protein digestibility and nitrogen balance

Items	C1	C2	E	SEM	P value
Excreted N, mg/100 g	0.422 ^{b}	0.331 ^{a}	0.285 ^{a}	0.017	0.0004
Absorbed N, mg/100 g	2.666 ^{b}	1.990 ^{a}	2.119 ^{a}	0.095	0.001
N digestibility, %	86.64 ^{a}	86.45 ^{a}	88.22 ^{a}	0.368	0.116
Excreted protein, %	2.632 ^{b}	2.064 ^{a}	1.776 ^{a}	0.103	0.0005
Absorbed protein, %	16.57 ^{b}	12.42 ^{a}	13.22 ^{a}	0.541	0.002
Protein digestibility, %	86.18 ^{a}	85.53 ^{a}	88.10 ^{a}	0.444	0.101

Values with different superscripts in the same row are significantly different ($P < 0.05$).

Prebiotics have the potential to alter gut microflora, influencing nitrogen metabolism, the quantity excreted, the ratio of fecal to urinary nitrogen, and the manner of ammonia emission from chicken manure (Such et al., 2023). Numerous tannins are regarded as sustainable feed additives because they originate from byproducts of plant-based agriculture and industry. For instance, chestnut tannins, which may be added to poultry feed, are extracted through the distillation of wood used in the construction industry (Mannelli et al., 2019).

While the inclusion of tannins in the feed of monogastric animals has been discouraged in the past due to their antinutrient properties, recent research indicates that with careful use, tannins can offer benefits to monogastric animals (Hassan et al., 2020). Smeriglio et al. (2017) suggested that the oak bark additive primarily consists of hydrolyzable tannins, with a minimal proportion of condensed tannins.

Condensed tannins have been recognized as inhibitors of various digestive enzymes, such as amylases, cellulases, pectinases, lipases, and proteases (Bhat et al., 2013). Their significant antinutritive impact can adversely affect the digestibility of lipids, starch, and amino acids (Garcia et al., 2004; Brestenský et al., 2012). Hammond et al. (2019) suggested that 3% oak bark powder is an appropriate content in poultry diets to enhance the birds' immunity and overall health without adverse effects.

Additionally, tannins have the potential to reduce the risk of livestock diseases and the spread of zoonotic pathogens. Furthermore, enhancement in performance may be a result of the beneficial influence of fostering a healthy intestinal ecosystem (Huang et al., 2018).

The mechanism by which tannins enhance growth in monogastric animals is not as well understood as it is in ruminants. One prevailing theory suggests that including tannins in low concentrations can boost feed intake and consequently improve the performance of monogastric animals (Huang et al., 2018). Moreover, while poultry possesses a distinct gastrointestinal tract compared to ruminants, tannins have the potential to modify gut health and microbiota in chickens, potentially enhancing nitrogen utilization and thereby reducing nitrogen emissions (Choi & Kim, 2020). In this study, the supplementation of a low-protein diet with oak bark led to a significant reduction of nitrogen excretion compared to the control and also a reduction of nitrogen by 14 % compared to the group with low dietary protein without supplementation with oak bark. This fact might suggest a reduction of nitrogen excretion due to the use of low-protein diets, and also a better utilization of dietary nitrogen due to the supplementation of diets with oak bark, as a source of tannins.

CONCLUSIONS

Low-protein diets can be considered one of the most effective strategies for reducing nitrogen excreta in laying hens. Moreover, enhancing the digestibility of dietary protein proves to be an effective approach in decreasing nitrogen excretion, for fostering sustainable poultry production. Results of this study showed that average egg weight significantly decreased in the groups with reduced dietary protein. The concentrations of nitrogen decreased by 21.7% in the group with a reduced dietary content of crude protein and 32.6 % in the group with a low protein diet and dietary oak bark supplementation, compared to the control group. This fact might suggest a reduction of nitrogen excretion due to the use of low-protein diets, and also a better utilization of dietary nitrogen due to the supplementation of diets with oak bark, as a source of tannins.

ACKNOWLEDGEMENTS

This research was funded by the Ministry of Research, Innovation, and Digitalization, Project PN 20.23-03.01, and the National Research Development Project to Finance Excellence (PFE)-8/2021.

REFERENCES

Alagawany, M., El-Hindawy, M. M., El-Hack, M. E., Arif, M., & El-Sayed, S. A. (2020). Influence of low-protein diet with different levels of amino acids on laying hen performance, quality and egg composition. *Anais da Academia Brasileira de Ciências*, *92*(1), 1-11.

Alfonso-Avila, A.R., Cirot, O., Lambert, W., & Létourneau-Montminy, M.P., 2022. Effect of low-protein corn and soybean meal-based diets on nitrogen utilization, litter quality, and water consumption in broiler chicken production: Insight from meta-analysis. *Animal*, *16*(3), 100458.

Aneja, V. P., Schlesinger, W. H., Nyogi, D., Jennings, G., Gilliam, W., Knighton, R. E., & Krishnan, S. (2006). Emerging national research needs for agricultural air quality. *Eos, Transactions American Geophysical Union*, *87*(3), 25-29.

Bagirov, V. A., Duskaev, G. K., Kazachkova, N. M., Rakhmatullin, S. G., Yausheva, E. V., Kosyan, D. B., & Dusaeva, K. B. (2018). Addition of Quercus cortex extract to broiler diet changes slaughter indicators and biochemical composition of muscle tissue. *Agricultural biology*, *53*(4), 799-810.

Bagno, O. A., Prokhorov, O. N., Shevchenko, S. A., Shevchenko, A. I., & Dyadichkina, T. V. (2018). Use of phytobioticts in farm animal feeding. *Agricultural biology*, *53*(4), 687-697.

Belloir, P., Lessire, M., Milgen, J. V., Schmidely, P., Corrent, E., & Tesseraud, S. (2015). Reducing dietary crude protein of broiler: a meta-analysis approach. *Actes des 11èmes Journées de la Recherche Avicole et Palmipèdes à Foie Gras*, 539-544.

Bhat, T.K., Kannan, A., Singh, B., & Sharma, O.P. (2013). Value addition of feed and fodder by alleviating the antinutritional effects of tannins. *Agricultural Research, 2*, 189–206.

Brestenský, M., Nitrayová, S., Patrás, P., & Heger, J. (2012) The quality of sorghum grain in aspect of utilization amino acids in pigs. *Journal of Microbiology and Biotechnology and Food Science*, 1, 1032-1039.

Choi, J., & Kim, W. K. (2020) Dietary application of tannins as a potential mitigation strategy for current challenges in poultry production: A review. *Animals*, 2020, *10*(12), 2389.

De Simon, B. F., Cadahia, E., Conde, E., & Garcia-Vallejo, M. C. (1996). Low molecular weight phenolic compounds in Spanish oak woods. *Journal of Agriculture and Food Chemistry*, *44*, 1507-1511.

Dróżdż, P., & Pyrzynska, K. (2018). Assessment of polyphenol content and antioxidant activity of oak bark extracts. *European Journal of Wood and Wood Products*, *76*, 793-795.

Dukhta, G., & Halas, V. (2023). Dynamic, Mechanistic Modeling Approach as a Tool to Mitigate N Excretion in Broilers. *Agriculture*, *13*(4), 808, 1-17.

Duskaev, G., Kvan, O., Vershinina, I., & Rakhmatullin, S. (2021). Assessment of lipid metabolism in broilers against plant extract and probiotic substance or their combined use. *Iranian Journal of Applied Animal Science*, *11*(1), 161-168.

Elansary, H. O., Szopa, A., Kubica, P., Ekiert, H., Mattar, M. A., Al-Yafrasi, M. A., & Yessoufou, K. (2019). Polyphenol profile and pharmaceutical potential of Quercus spp. bark extracts. *Plants*, *8*(11), 486, 1-14.

Englmaierová, M., Skřivan, M., Taubner, T., & Skřivanová, V. (2022). Increasing eggshell strength and fat-soluble vitamins content in yolk by including chestnut wood tannin in polyunsaturated fatty acid-enriched diet of young hens. *Italian Journal of Animal Science*, *21*(1), 1343-1351.

Fisinin, V. I., Ushakov, A. S., Duskaev, G. K., Kazachkova, N. M., Nurzhanov, B. S., Rakhmatullin, S. G., & Levakhin, G. I. (2018). Mixtures of biologically active substances of oak bark extracts change immunological and productive indicators of broilers. *Agricultural biology*, *53*(2), 385-92.

Garcia, R. G., Mendes, A. A., Sartori, J. R., de Lima Almeida Paz, I. C., Takahashi, S. E., Pelícia, K., Komiyama, C. M., & Quinteiro, R. R. (2004)

Digestibility of feeds containing sorghum, with and without tannin, for broiler chickens submitted to three room temperatures. *Brazilian Journal of Poultry Science*, *6*, 55–60.

Gautron, J., Réhault-Godbert, S., Van de Braak, T. G. H., & Dunn, I. C. (2021) What are the challenges facing the table egg industry in the next decades and what can be done to address them? *Animal*, *15*, 100282.

Hammod, A. J., Alshukri, A. Y., Areaaer, A. H., Alfertosi, K. A., & Alyasari, A. F. (2019). Research article the effect of adding oak bark powder to the diet on some productive and immunological characteristics of broiler chicks. *International Journal of Poultry Science*, *18*(1), 7-13.

Hassan, Z. M., Manyelo, T. G., Selaledi, L., & Mabelebele, M. (2020). The effects of tannins in monogastric animals with special reference to alternative feed ingredients. *Molecules*, *25*(20), 4680, 1-17.

Heo, Y. J., Park, J., Kim, Y. B., Kwon, B. Y., Kim, D. H., Song, J. Y., & Lee, K. W. (2023) Effects of dietary protein levels on performance, nitrogen excretion, and odor emission of growing pullets and laying hens. *Poultry Science*, *102*(8), 102798.

Huang, Q., Liu, X., Zhao, G., Hu, T., & Wang, Y. (2018) Potential and challenges of tannins as an alternative to in-feed antibiotics for farm animal production. *Animal Nutrition*, *4*, 137–150.

Ji, F., Fu, S. Y., Ren, B., Wu, S. G., Zhang, H. J., Yue, H. Y., & Qi, G. H. (2014). Evaluation of amino-acid supplemented diets varying in protein levels for laying hens. *Journal of Applied Poultry Research*, *23*(3), 384-392.

Kerr, B. J. (1995) *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, 47–68.

Li, M., Feng, L., Jiang, W. D., Wu, P., Liu, Y., Jiang, J., Kuang, S. Y., Tang, L., & Zhou, X. Q. (2020) Condensed tannins decreased the growth performance and impaired intestinal immune function in on-growing grass carp (*Ctenopharyngodon idella*). *British Journal of Nutrition*, *123*(7), 737-755.

Malomo, G. A., Bolu, S. A., Madugu, A. S., & Usman, Z. S. (2018) Nitrogen Emissions and Mitigation Strategies in Chicken Production. *Animal Husbandry and Nutrition*, *43*, 43–62.

Mannelli, F., Team, H., Tosi, G., Secci, G., Daghio, M., Massi, P., Fiorentini, L. Galigani, I. Lancini, S. & Rapaccini, S. (2019) Effect of Chestnut Tannins and Short Chain Fatty Acids as Anti-Microbials and as Feeding Supplements in Broilers Rearing and Meat Quality. *Animals*, *9*(9), 659, 1-15.

Santonja, G. G., Georgitzikis, K., Scalet, B. M., Montobbio, P., Roudier, S., & Sancho, L. D. (2017). Best available techniques (BAT) reference document for the intensive rearing of poultry or pigs. *EUR 28674 EN*, 11, 898.

Selle, P. H., Cadogan, D. J., Li, X., & Bryden, W. L. (2010). Implications of sorghum in broiler chicken nutrition. *Animal Feed Science and Technology*, *156*(3-4), 57–74.

Serrano, J., Puupponen-Pimiä, R., Dauer, A., Aura, A. M., & Saura-Calixto, F. (2009). Tannins: current knowledge of food sources, intake, bioavailability and biological effects. *Molecular nutrition & food research*, *53*(S2), S310-S329.

Sirirat, N., Lu, J. J., Hung, A. T. Y., & Lien, T. F. (2013) Effect of different levels of nanoparticles chromium picolinate supplementation on performance, egg quality, mineral retention, and tissues minerals accumulation in layer chickens. *Journal of Agricultural Science*, *5*, 150-159.

Smeriglio, A., Barreca, D., Bellocco, E., & Trombetta, D. (2017) Proanthocyanidins and hydrolysable tannins: Occurrence, dietary intake and pharmacological effects. *British Journal of Pharmacology*, *174*(11), 1244-1262.

Soomro, R. N., Yao, J., El-Hack, M. E. A., Arain, M. A., Abbasi, I. H. R., Saeed, M., & Tufarelli, V. (2018). Significance of endogenous amino acid losses in the nutrition of some poultry species: a review. *JAPS: Journal of Animal & Plant Sciences*, *28*(6), 1547-1557.

Such, N., Mezőlaki, Á., Rawash, M. A., Tewelde, K. G., Pál, L., Wágner, L., & Dublecz, K. (2023). Diet Composition and Using Probiotics or Symbiotics Can Modify the Urinary and Faecal Nitrogen Ratio of Broiler Chicken's Excreta and Also the Dynamics of In Vitro Ammonia Emission. *Animals*, *13*(3), 332.

Summers, J. D., & Leeson, S. (1994). Laying hen performance as influenced by protein intake to sixteen weeks of age and body weight at point of lay. *Poultry Science*, *73*(4), 495-501.

Windisch, W., & Kroismayr, A. (2006). The effects of phytobiotics on performance and gut function in monogastrics. *World nutrition forum: The future of animal nutrition*, 85-90.