Poultry Studies, 23(2), xx-xx
http://doi.org/xxxxxx/jpr.xxxxxxx

EARLY VIEW

RESEARCH PAPER

Fresh Forage Acceptance, Egg Production, and Egg Quality in Lohmann White Laying Hens Offered Fresh Chicory, Ryegrass, or Alfalfa

Nicolás Portales[1], Juan Pablo Keim[1], Mónica Gandarillas[1, *]

[1] Universidad Austral de Chile, Institute of Animal Production, Valdivia, Chile

Article History
Received May 18, 2026
Accepted: Jun 18, 2026
First Online:

***Corresponding Author**
Tel: + 569 85273098
E-mail: monica.gandarillas@uach.cl

Keywords
Dietary supplementation
Feed refusal
Yolk pigmentation
Albumen quality
Cage-free systems

Abstract

This study evaluated fresh forage acceptance, egg production, and egg quality in 16 Lohmann White hens assigned to four treatments in a 4 × 4 Latin square design. Forage treatments contained 75% concentrate and 25% fresh forage on a dry matter basis. Feed offered and residues were recorded daily to estimate apparent acceptance. Chicory had lower residual dry matter than ryegrass or alfalfa ($P < 0.0001$). Egg production ($P = 0.47$) and egg quality traits ($P > 0.05$) did not differ among treatments. Findings suggest greater apparent acceptance of chicory, but remain preliminary.

Introduction

Alternative egg production systems have expanded in response to growing consumer concern about animal welfare. Cage-free and free-range systems allow laying hens to express natural behaviors, including foraging, dust bathing, and social interaction (Hartcher and Jones, 2017; Lay et al., 2011; Sinclair et al., 2022). In these systems, access to forage may contribute to behavioral enrichment and may influence egg quality traits, particularly yolk color, through the intake of natural pigments such as carotenoids and xanthophylls present in green plants (Hammershøj and Johansen, 2016; Mugnai et al., 2014). Egg quality is a key attribute for consumers and producers. External traits such as shell cleanliness, egg weight, egg shape, and shell thickness affect marketability and handling, whereas internal traits including yolk color, albumen height, yolk index, and Haugh units are associated with freshness and consumer acceptance (Roberts, 2004; Silversides and Budgell, 2004). Access to pasture or forage has been reported to affect yolk pigmentation and some shell traits, although many egg quality characteristics are strongly influenced by hen age, genotype, nutrition, and environmental conditions (Aygun et al., 2024; Silversides and Scott, 2001). Fresh forages such as chicory, ryegrass, and alfalfa are candidate supplements for alternative laying hen systems (Aygun et al., 2024; Hammershøj and Johansen, 2016). Chicory (*Cichorium intybus*) is a broadleaf forage with high water content, high palatability, and functional compounds such as

carotenoids and phenolics (Barry, 1998; Montefusco et al., 2015; Zheng et al., 2019). In poultry, fresh chicory inclusion has been associated with effects on performance, meat and egg quality, and intestinal microbiota (Zheng et al., 2019). Its broadleaf morphology may also make it more accessible to hens than narrow-leaved grasses.

Ryegrass (*Lolium perenne*) is widely used in temperate pasture systems because of its productivity, digestibility, and grazing tolerance (Guy et al., 2018; Wilkins, 1991). Alfalfa (*Medicago sativa*) is a protein-rich legume and an important source of carotenoids, particularly lutein and other xanthophylls that may contribute to yolk pigmentation when consumed in sufficient amounts (Elgersma et al., 2013; Scott et al., 1968). However, information on the acceptance of fresh chicory, ryegrass, and alfalfa by laying hens under controlled cage-free conditions remains limited.

The objective of this study was to evaluate fresh forage acceptance, egg production, and egg quality traits in Lohmann White laying hens offered diets supplemented with fresh chicory, ryegrass, or alfalfa. We hypothesized that chicory would show greater apparent acceptance than ryegrass or alfalfa and would increase yolk color without negatively affecting other egg quality traits.

Materials and Methods

Experimental site and animals

The experiment was conducted at the Estación Experimental Agropecuaria Austral, Universidad Austral de Chile, located near Valdivia, Los Ríos Region, Chile (39°48'S, 73°15'W). Hens were housed in the monogastric laboratory facilities, and egg quality analyses were performed at the Animal Nutrition Laboratory of the Institute of Animal Production, Universidad Austral de Chile.

Sixteen Lohmann White laying hens aged 37 to 40 weeks were used. Hens were housed in eight pens of two hens each. Pens were randomly assigned to four groups, with each group consisting of two pens. The pen was the experimental unit, giving four replicate pens per treatment across the experiment. A 16-h photoperiod was maintained using natural and supplemental artificial light, according to management guidelines for this breed (Lohmann Breeders, 2022).

Temperature and relative humidity were not continuously recorded inside the experimental room. However, the trial coincided with an unusually warm period in southern Chile. The Dirección Meteorológica de Chile reported that February 2023 was among the warmest February months recorded in central and southern Chile, with heat-wave events extending from the Metropolitan Region to Los Ríos Region and a 7-d heat-wave event in Valdivia (Dirección Meteorológica de Chile, 2023). Therefore, environmental conditions were considered when interpreting period effects, although the absence of indoor temperature and humidity measurements is a limitation of the study.

Experimental design and treatments

The trial lasted four weeks, from 6 February to 2 March 2023. Treatments were allocated using a 4 × 4 Latin square arrangement, with four treatment groups and four experimental periods. Each group received each treatment once during the experiment. Period and group were included in the design to account for temporal variation and group differences. The treatment allocation is presented in Supplementary Table 2.

Four treatments were evaluated: control, ryegrass, alfalfa, and chicory. The control treatment consisted of 100% commercial concentrate. The forage treatments consisted of 75% commercial concentrate and 25% fresh forage on a dry matter basis. The forage sources were fresh ryegrass, fresh alfalfa, and fresh chicory.

The commercial concentrate contained 15.0% crude protein, 2.5% ether extract, 8.5% crude fiber, 3.0% calcium, and 10.0% moisture. Diets were formulated considering an expected dry matter intake of 108 g per hen per day, based on the recommended feed intake range for Lohmann laying hens (Lohmann Breeders, 2022). In the forage treatments, each pen received 162 g dry matter from concentrate and 54 g dry matter from forage. On a fresh matter basis, the daily forage offer was 540 g chicory, 270 g ryegrass, or 216 g alfalfa, together with 180 g concentrate. The control treatment received 240 g concentrate per pen per day.

Fresh matter amounts were calculated considering approximate moisture contents of 90% for chicory, 80% for ryegrass, and 75% for alfalfa (Guy et al., 2018; Momberg et al., 2017; Demanet, 2019). The forage batches used in this experiment were not chemically analyzed. Therefore, reference nutritional and functional information for the forage species is provided in Supplementary Tables 1 and 3 only to support interpretation.

Feeding management and apparent forage acceptance

Diets were offered according to the Latin square treatment allocation. Each period lasted one week. Feeding of the assigned treatment started on Sunday, one day before the first egg collection used for analysis, because approximately 24 h are required for egg formation. Eggs collected from Monday to Thursday were used for the experimental evaluation. No formal washout period was included between treatments; therefore, potential carry-over effects cannot be completely excluded. To reduce this limitation, period and treatment × period effects were included in the statistical model. Fresh forage acceptance and apparent intake were estimated daily from the difference between feed offered and feed residues. The remaining forage

and concentrate from the previous day were collected and weighed daily, and residues were expressed on a dry matter basis. Lower residue was interpreted as greater apparent acceptance of the diet. However, this method does not provide individual intake and does not separate true refusal from possible feed wastage; therefore, the results are reported as apparent acceptance rather than precise individual nutrient intake.

Fresh forages were offered as harvested fresh material. Differences in leaf structure and physical bulk among chicory, ryegrass, and alfalfa were therefore part of the dietary comparison. Particle size was not standardized among forage species, which is acknowledged as a limitation because forage form may influence acceptance and intake.

Egg production and egg collection

Egg production was recorded throughout the experimental period. Eggs were collected daily; however, only eggs collected from Monday to Thursday within each experimental period were used for quality analyses. A total of 111 eggs were collected and evaluated during the experiment.

External egg quality

External egg quality traits included shell cleanliness, egg weight, egg length, egg width, shape index, and shell thickness. Shell cleanliness was assessed using a visual score from 1 to 5, where 1 indicated a clean egg and higher scores indicated greater shell dirtiness. Egg weight was recorded in grams. Egg length, egg width, and shell thickness were measured using an electronic caliper. Shape index was calculated as:

$$\text{Shape index} = (\text{egg width} / \text{egg length}) \times 100$$

Internal egg quality

Internal egg quality traits included yolk color, yolk index, albumen height, and Haugh units. Yolk color was assessed using the Roche yolk color scale, ranging from 1, pale yellow, to 15, dark orange. Yolk height and yolk diameter were measured using an electronic caliper, and yolk index was calculated as:

$$\text{Yolk index} = (\text{yolk height} / \text{yolk diameter})$$

Albumen height was measured and used together with egg weight to calculate Haugh units according to Eisen et al. (1962):

$$HU = 100 \times \log10(h - 1.7w^{0.37} + 7.6)$$

where HU is Haugh units, h is albumen height, and w is egg weight.

Statistical analysis

Data were analyzed by analysis of variance using the MIXED procedure of SAS (SAS Institute Inc., Cary, NC). The experimental unit was the pen. The model included treatment, period, and the treatment × period interaction as fixed effects, and pen as a random effect. The statistical model was:

$$Y_{ijk} = \mu + T_i + P_j + (T \times P)_{ij} + G_k + e_{ijk},$$

where Y_{ijk} is the observed response, μ is the overall mean, T_i is the fixed effect of treatment, P_j is the fixed effect of period, $(T \times P)_{ij}$ is the fixed interaction between treatment and period, G_k is the random effect of group, and e_{ijk} is the residual error. Egg production and feed residues were analyzed using pen-period means as the unit of observation. The remaining egg quality variables were recorded on individual eggs; because the experimental unit was the pen, the corresponding tests of treatment effects should be interpreted with caution, although no treatment effect on these traits was detected. Differences were considered significant at $P < 0.05$. Least-squares means were compared using the Tukey-Kramer adjustment when treatment effects were significant. Results are presented as least-squares means ± standard error.

Results and Discussion

Residual dry matter differed among dietary treatments ($P < 0.0001$; Table 4). The control treatment had the lowest residue (4.21 ± 1.35 g DM), reflecting near-complete concentrate consumption. Among the forage treatments, chicory residue (27.79 ± 4.90 g DM) was lower than that of ryegrass (32.67 ± 9.95 g DM) or alfalfa (38.37 ± 7.27 g DM). Residual dry matter did not differ among experimental periods ($P = 0.1486$). These results indicate greater apparent acceptance of chicory than ryegrass or alfalfa.

The greater apparent acceptance of chicory is consistent with its broadleaf structure and reported palatability. Laying hens have been observed to show greater preference for broadleaf species than for grasses or legumes (Gandarillas et al., 2025). Chicory also contains bioactive compounds and has been associated with positive effects on growth performance, egg quality, meat quality, and intestinal microbiota when included in poultry diets (Zheng et al., 2019). In the present study, the lower residue in the chicory treatment suggests greater apparent voluntary consumption of this forage relative to ryegrass and alfalfa, although feed wastage could not be separated from refusal. The high moisture content of fresh forages resulted in substantially greater fresh matter offers than the control diet. Hens in the control treatment received 240 g of concentrate per pen per day, whereas hens in the forage treatments received 180 g concentrate plus 540 g fresh chicory, 270 g fresh ryegrass, or 216 g fresh alfalfa per pen per day.

Although diets were formulated on an equal dry matter basis, the physical bulk of these fresh offers may have constrained effective consumption of the complete ration.

Egg production did not differ among treatments when analyzed at the pen level (P = 0.47; Table 4), with means of 8.00 +/- 1.63, 7.00 +/- 2.94, 6.75 +/- 2.04, and 6.25 +/- 1.27 eggs per pen per collection period for control, chicory, ryegrass, and alfalfa, respectively. Egg production did differ among periods (P = 0.0415), being highest in period 1 and lowest in period 3.

Although egg production was numerically lower in the forage treatments than in the control, this difference was not statistically significant at the pen level and should not be interpreted as a treatment effect. Any numerical reduction may reflect incomplete consumption of the offered ration and the high fresh matter volume of forage diets, but because nutrient intake was not directly measured and feed wastage could not be separated from refusal, this interpretation remains speculative. The significant period effect may also have been influenced by environmental conditions. The experiment coincided with an unusually warm February in southern Chile, and the Dirección Meteorológica de Chile reported heat-wave events during February 2023, including a 7-d event in Valdivia (Dirección Meteorológica de Chile, 2023). Heat stress can reduce feed intake and egg production in laying hens (Mashaly et al., 2004).

Most external egg quality traits were unaffected by dietary treatment. Shell cleanliness, egg weight, egg length, egg width, shape index, and shell thickness did not differ significantly among treatments (P > 0.05; Table 4). Mean egg weight ranged from 58.90 to 59.75 g and shell thickness averaged 0.44 mm across all treatments. The absence of treatment effects on egg weight and egg dimensions is consistent with reports that these traits are governed mainly by hen body weight and flock age rather than by short-term dietary changes (Fletcher et al., 1983; Garcia et al., 2016; Romera et al., 2022). These results is 1983 indicate that short-term inclusion of fresh forage did not compromise the main external quality characteristics of eggs.

Treatment × period interactions were detected for egg weight (P = 0.0108), egg length (P = 0.0002), and egg width (P = 0.0073), and shell thickness was affected by period (P = 0.0006). These period effects may reflect changes in environmental conditions, hen physiological state, or short-term variation in feed intake. Shell quality is influenced by genetic, nutritional, environmental, and age-related factors (Samiullah et al., 2014), and heat stress can impair shell traits through reduced feed intake and altered mineral metabolism (Mashaly et al., 2004). Importantly, the absence of a main treatment effect indicates that forage source did not systematically alter external egg quality traits.

Internal egg quality traits were similarly unaffected by dietary treatment. Yolk color did not differ among treatments (P = 0.4834; Table 4), with Roche scale values ranging from 8.77 in the control treatment to 9.27 in the chicory treatment. Yolk index, albumen height, and Haugh units were also unaffected by treatment (P > 0.05). These results do not support the hypothesis that chicory supplementation would improve yolk color relative to ryegrass, alfalfa, or concentrate alone under the conditions of this trial.

The absence of a treatment effect on yolk color contrasts with studies reporting enhanced yolk pigmentation in hens with access to pasture or forage, attributed to intake of carotenoids and xanthophylls from green plants (Hammershøj and Johansen, 2016; Kojima et al., 2022; Mugnai et al., 2014). Chicory and alfalfa contain lutein and other carotenoids that can contribute to yolk pigmentation when consumed in sufficient quantities (Elgersma et al., 2013; Rapisarda et al., 2023). In the present study, apparent forage intake may have been insufficient to meaningfully increase carotenoid deposition in the yolk over the four-week experimental period.

Albumen height and Haugh units were influenced by period but not by treatment. Haugh units, calculated from albumen height and egg weight, are widely used as indicators of internal egg quality (Eisen et al., 1962) and are sensitive to egg storage conditions, hen age, genotype, and environmental temperature (Rizzi, 2021; Silversides and Budgell, 2004; Silversides and Scott, 2001). The period effects observed here are therefore more plausibly attributed to short-term fluctuations in environmental conditions or egg quality dynamics than to any sustained influence of dietary forage.

Overall, fresh chicory showed greater apparent acceptance than ryegrass and alfalfa, yet fresh forage supplementation did not improve any measured egg quality trait. The principal practical limitation of the forage treatments was the large fresh matter volume required to deliver the target dry matter intake. Given the small number of hens, short duration, lack of direct nutrient intake measurements, and absence of indoor temperature and humidity records, the findings should be regarded as preliminary. Future studies should evaluate reduced forage inclusion levels, chopped or processed forage forms, larger flocks, and longer experimental periods to fully assess the potential of fresh forage supplementation in cage-free or free-range laying hen systems.

Conclusion

In this preliminary study, fresh chicory was more readily accepted by Lohmann White laying hens than fresh ryegrass or alfalfa, based on lower feed residues and apparent intake estimated at the pen level. Short-term fresh forage supplementation did not improve yolk color or other external and internal egg quality traits, and egg production did not differ significantly among treatments. Fresh forage can therefore be offered without compromising egg production or egg quality under the conditions of this trial, although practical use

requires attention to forage form, moisture content, physical bulk, and environmental conditions. Further studies with larger flocks, longer duration, and direct nutrient intake measurements are needed.

References

Aygun, A., Narinc, D., Ozkose, A., Arisoy, H., Acar, R., Uzal, S., & Bulut, C. (2024). Effects of outdoor plant varieties on performance, egg quality, behavior, and economic analysis of Turkey local chicken from 20 to 36 weeks of age. *Journal of the Hellenic Veterinary Medical Society, 75*(1), 7007-7018. https://doi.org/10.12681/jhvms.33984

Barry, T. N. (1998). The feeding value of chicory (Cichorium intybus) for ruminant livestock. *Journal of Agricultural Science, 131*(3), 251-257. https://doi.org/10.1017/S002185969800584X

Demanet, R. (2019). Manual de especies forrajeras (3rd ed.). Watt's, CORFO, and Universidad de La Frontera, Concepción, Chile.

Dirección Meteorológica de Chile. (2023). *El Clima Hoy: Récord de altas temperaturas en febrero* [The climate today: Record high temperatures in February]. Oficina de Servicios Climáticos, Santiago, Chile. Issued 27 February 2023. https://climatologia.meteochile.gob.cl/

Eisen, E. J., Bohren, B. B., & McKean, H. E. (1962). The Haugh unit as a measure of egg albumen quality. *Poultry Science, 41*(5), 1461-1468. https://doi.org/10.3382/ps.0411461

Elgersma, A., Søegaard, K., & Jensen, S. K. (2013). Fatty acids, alpha-tocopherol, beta-carotene, and lutein contents in forage legumes, forbs, and a grass-clover mixture. *Journal of Agricultural and Food Chemistry, 61*(49), 11913-11920. https://doi.org/10.1021/jf403195v

Fletcher, D. L., Britton, W. M., Pesti, G. M., Rahn, A. P., & Savage, S. I. (1983). *Poultry Science*, 62(9), 1800–1805. Update the in-text citation too.

Gandarillas, M., Elizalde, R., Irazabal, S., Calvache, I., Keim, J. P., & Balocchi, O. (2025). Research note: A pilot study comparing White Leghorn and Isa Brown breeds under a free-range system on performance, egg quality and pasture characteristics. *Poultry Science, 104*(7), 105188. https://doi.org/10.1016/j.psj.2025.105188

Gandarillas, M., Lopez, I. F., Dorner, J., Tadich, T., Balocchi, O., & Guarda, C. (2026). Pasture species selection for free-range egg production systems: Implications for laying performance, egg quality, hen welfare, and soil functioning. *Poultry Science*, 107269. https://doi.org/10.1016/j.psj.2026.107269

Garcia, D., Colas, M., Lopez, W., Perez, O., Sanchez, A., Lamazares, M., & Grandia, R. (2016). El peso corporal y su efecto sobre indicadores bioproductivos en gallinas White Leghorn L33. *Revista de la Facultad de Medicina Veterinaria y de Zootecnia, 63*(3), 188-200. https://doi.org/10.15446/rfmvz.v63n3.62714

Guy, C., Hennessy, D., Gilliland, T. J., Coughlan, F., McClearn, B., Dineen, M., & McCarthy, B. (2018). Comparison of perennial ryegrass (Lolium perenne L.) ploidy and white clover (Trifolium repens L.) inclusion for herbage production, utilization and nutritive value. *Grass and Forage Science, 73*, 865-877. https://doi.org/10.1111/gfs.12366

Hammershøj, M., Johansen, N. F. (2016). Review: The effect of grass and herbs in organic egg production on egg fatty acid composition, egg yolk colour and sensory properties. *Livestock Science, 194*, 37-43. https://doi.org/10.1016/j.livsci.2016.11.001

Hartcher, K. M., & Jones, B. (2017). The welfare of layer hens in cage and cage-free housing systems. *World's Poultry Science Journal, 73*(4), 767-782. https://doi.org/10.1017/S0043933917000812

Kojima, S., Koizumi, S., Kawami, Y., & Osawa, A. (2022). Effect of dietary carotenoid on egg yolk color and singlet oxygen quenching activity of laying hens. *The Journal of Poultry Science, 59*(2), 137-142. https://doi.org/10.2141/jpsa.0210032

Lay, D. C., Jr., Fulton, R. M., Hester, P. Y., Karcher, D. M., Kjær, J. B., Mench, J. A., Mullens, B. A., Newberry, R. C., Nicol, C. J., O'Sullivan, N. P., & Porter, R. E. (2011). Hen welfare in different housing systems. *Poultry Science, 90*(1), 278-294. https://doi.org/10.3382/ps.2010-00962

Lohmann Breeders. (2022). Lohmann LSL-Classic: Management guide. Lohmann Breeders.

Mashaly, M. M., Hendricks, G. L., Kalama, M. A., Gehad, A. E., Abbas, A. O., & Patterson, P. H. (2004). Effect of heat stress on production parameters and immune responses of commercial laying hens. *Poultry Science, 83*(6), 889-894. https://doi.org/10.1093/ps/83.6.889

Momberg, J., Balocchi, O. A., & Keim, J. P. (2017). Evaluacion del establecimiento de tres cultivares de achicoria (Cichorium intybus L.) en siembra asociada a ballica bianual y avena en la comuna de Purranque. *Agro Sur, 45*(2), 21-29. https://doi.org/10.4206/agrosur.2017.v45n2-03

Montefusco, A., Semitaio, G., Marrese, P. P., Lurlaro, A., De Caroli, M., Piro, G., Dalessandro, G., & Lenucci, M. S. (2015). Antioxidants in varieties of chicory (Cichorium intybus L.) and wild poppy (Papaver rhoeas L.) of Southern Italy. Journal of Chemistry*, 2015*, 923142. https://doi.org/10.1155/2015/923142.

Mugnai, C., Sossidou, E. N., Dal Bosco, A., Ruggeri, S., Mattioli, S., & Castellini, C. (2014). The effects of husbandry system on the grass intake and egg quality of laying hens. Journal of the Science of Food and Agriculture, 94, 459-467. https://doi.org/10.1002/jsfa.6269

Rapisarda, S., O'Neill, G., & Abu-Ghannam, N. (2023). Fatty acid, carotenoid and fat-soluble vitamin composition of multispecies swards grown in Ireland: Implications for a sustainable feed in dairy farming. *Dairy, 4*(2), 300-315. https://doi.org/10.3390/dairy4020021

Rizzi, C. (2021). Albumen quality of fresh and stored table eggs: Hen genotype as a further chance for consumer choice. *Animals, 11*(1), 135. https://doi.org/10.3390/ani11010135

Roberts, J. R. (2004). Factors affecting egg internal quality and egg shell quality in laying hens. *The Journal of Poultry Science, 41*, 161-177. https://doi.org/10.2141/jpsa.41.161

Romera, E., Advinculo, S., Canet, Z., Dottavio, A., & Di Masso, R. (2022). Dinamica del largo, el ancho y la forma del huevo en tres poblaciones de gallinas camperas. InVet, 24(2), 1-9.

Samiullah, S., Roberts, J. R., & Chousalkar, K. (2014). Effect of production system and flock age on egg quality and total bacterial load in commercial laying hens. *The Journal of Applied Poultry Research, 23*(1), 59-70. https://doi.org/10.3382/japr.2013-00805

Scott, M. L., Ascarelli, I., & Olson, G. (1968). Studies of egg pigmentation. Poultry Science*, 47*, 863-872. https://doi.org/10.3382/ps.0470863

Silversides, F. G., & Budgell, K. (2004). The relationships among measures of egg albumen height, pH, and whipping volume. Poultry Science, *83*(10), 1619-1623. https://doi.org/10.1093/ps/83.10.1619

Silversides, F. G., & Scott, T. A. (2001). Effect of storage and layer age on quality of eggs from two lines of hens. *Poultry Science, 80*(8), 1240-1245. https://doi.org/10.1093/ps/80.8.1240

Sinclair, M., Lee, N. Y. P., Hötzel, M. J., de Luna, M. C. T., Sharma, A., Idris, M., Islam, M. A., Iyasere, O. S., Navarro, G., Ahmed, A. A., Curry, M., Burns, G. L., & Marchant, J. N. (2022). Consumer attitudes towards egg production systems and hen welfare across the world. Frontiers in Animal Science*, 3*, 995430. https://doi.org/10.3389/fanim.2022.995430

Wilkins, P. W. (1991). Breeding perennial ryegrass for agriculture. *Euphytica, 52*, 201-214. https://doi.org/10.1007/BF00029397

Zheng, M., Mao, P., Tian, X., Meng, L., & Li, Y. (2019). Growth performance, carcass characteristics, meat and egg quality, and intestinal microbiota in Beijing-you chicken on diets with inclusion of fresh chicory forage. *Italian Journal of Animal Science, 18*(1), 1310-1320. https://doi.org/10.1080/1828051X.2019.1643794

Table 1. Functional and nutritional information for the fresh forage species offered to laying hens (Supplementary material)

Forage species	Functional group	Crude protein	Key structural and functional traits	Evidence basis
Chicory *(Cichorium intybus)*	Perennial broadleaf herb	20–25% DM depending on phenological stage	Deep taproot and large accessible leaves; crown sensitivity possible. High pigmentation potential reported in poultry studies. Contains antioxidant compounds and bioactive metabolites such as condensed tannins, phenolics, and sesquiterpene lactones.	Poultry evidence available for intake and egg traits; persistence evidence limited.
Ryegrass *(Lolium perenne)*	Temperate perennial grass	15–25% DM depending on phenological stage	Dense tillering and rapid regrowth; fiber increases rapidly with maturity. Moderate pigment potential, especially in leafy vegetative tissue.	Poultry evidence indirect; strong agronomic evidence.
Alfalfa *(Medicago sativa)*	Perennial legume	20–25% DM depending on phenological stage	Fresh green leaves provide alpha-linolenic acid and tocopherols. Erect growth and crown sensitivity to repeated damage. High carotenoid content; good source of alpha-linolenic acid precursors; rich in tocopherols, saponins, flavonoids, and phenolics.	Poultry diet evidence exists; grazing persistence evidence limited.

Note: Information was adapted from Gandarillas et al. (2026). Values should be interpreted as approximate because forage composition varies with cultivar, phenological stage, season, soil fertility, and management. These values are provided as reference information and do not represent chemical analyses of the specific forage batches used in the present experiment. Botanical images were adapted from Gandarillas et al. (2026).

Table 2. Treatment allocation according to the 4 × 4 Latin square design (Supplementary material)

Group	Period 1	Period 2	Period 3	Period 4
Group 1	Control	Ryegrass	Chicory	Alfalfa
Group 2	Ryegrass	Alfalfa	Control	Chicory
Group 3	Alfalfa	Chicory	Ryegrass	Control
Group 4	Chicory	Control	Alfalfa	Ryegrass

Note: Control = 100% commercial concentrate; forage treatments = 75% commercial concentrate + 25% fresh forage on a dry matter basis. Periods corresponded to experimental weeks.
Source: Latin square allocation based on the experimental protocol; nutritional and botanical information adapted from Gandarillas et al. (2026).

Table 3. Reference values for dry matter, crude protein, and carotenoid content of the fresh forage species, compiled from the literature (Supplementary material)

Forage species	Dry matter (approx. moisture)	Crude protein (% DM)	Lutein (mg/kg DM)	Total carotenoids (mg/kg DM)
Chicory (*Cichorium intybus)*	~10% DM (~90% moisture) (Momberg et al., 2017)	20–25	~150 (Elgersma et al., 2013)	Not available
Ryegrass (*Lolium perenne*)	~20% DM (~80% moisture) (Guy et al., 2018)	15–25	24–56 (Rapisarda et al., 2023)	Not available
Alfalfa (*Medicago sativa*)	~25% DM (~75% moisture) (Demanet, 2019)	20–25	~125 (Elgersma et al., 2013)	400–500 (Scott et al., 1968)

Note: DM = dry matter. Values are approximate reference figures compiled from published literature and do not represent chemical analyses of the specific forage batches used in the present experiment, which were not analyzed. Composition varies with cultivar, phenological stage, season, soil fertility, and management. Crude protein ranges correspond to those reported in Supplementary Table 1.

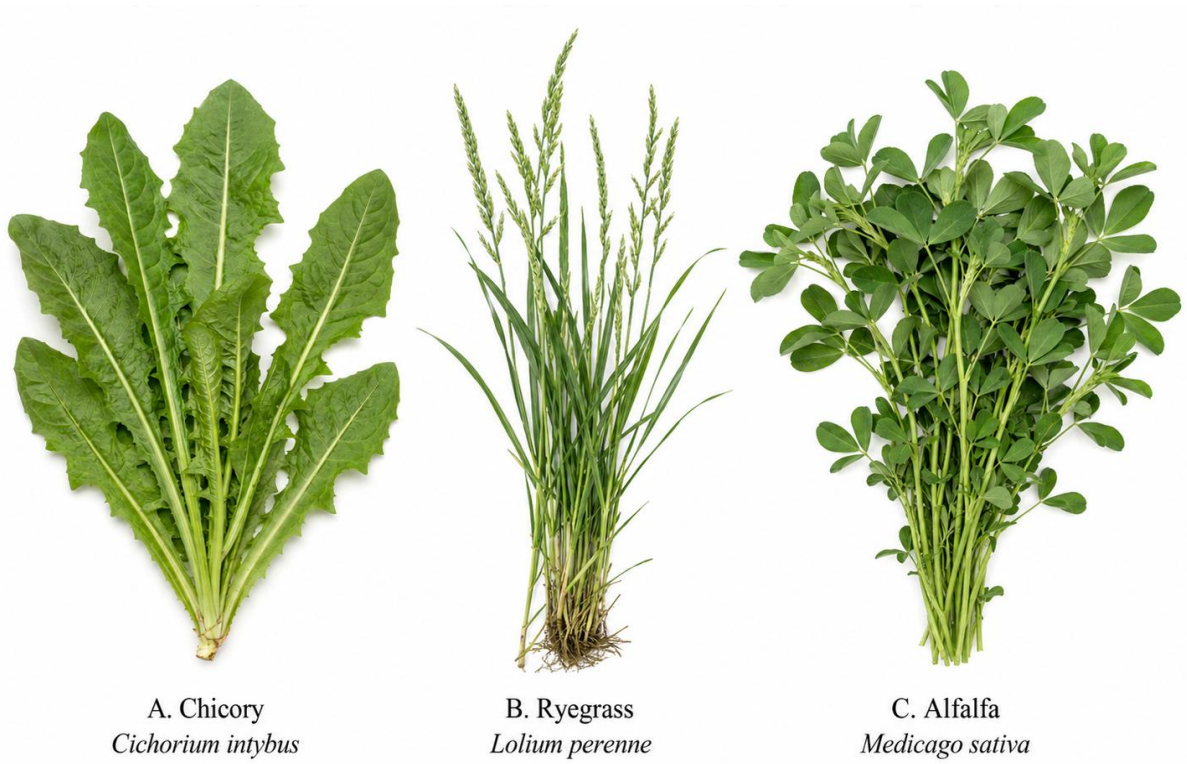

Figure 1. Fresh forage species offered to laying hens during the experiment. A. Chicory (*Cichorium intybus*); B. Ryegrass (*Lolium perenne*); C. Alfalfa (*Medicago sativa*). Botanical images adapted from Gandarillas et al. (2026).

Table 4. Effect of dietary treatment on residue, egg production, and selected egg quality traits in Lohmann White laying hens offered fresh forages

Variable	Concentrate	Ryegrass	Alfalfa	Chicory	P-value
Residual DM (g)	4.21^{c} ± 1.35	32.67^{a} ± 9.95	38.37^{a} ± 7.27	27.79^{b} ± 4.90	<0.0001
Egg production (mean eggs)	8.00 ± 1.63	6.75 ± 2.04	6.25 ± 1.27	7.00 ± 2.94	0.4700
Egg weight (g)	58.90 ± 1.03	59.75 ± 1.10	59.24 ± 1.11	59.17 ± 1.13	0.9026
Shell thickness (mm)	0.44 ± 0.01	0.44 ± 0.01	0.44 ± 0.01	0.44 ± 0.01	0.9949
Yolk color	8.77 ± 0.34	9.26 ± 0.37	9.15 ± 0.37	9.27 ± 0.38	0.4834
Albumen height (mm)	7.78 ± 0.49	7.33 ± 0.51	7.85 ± 0.51	7.54 ± 0.52	0.4908
Yolk index	0.42 ± 0.01	0.39 ± 0.01	0.39 ± 0.01	0.40 ± 0.01	0.1736
Haugh unit	87.71 ± 3.31	84.15 ± 3.46	87.79 ± 3.48	86.05 ± 3.49	0.4726

Values are presented as least-squares means ± standard error. DM: dry matter. Within a row, means with different superscript letters differ at $P < 0.05$. Superscript letters are shown only for variables with significant treatment effects.