

## The identification of low-producing hens in egg production systems using objective methods

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### Abstract

In commercial egg production, hen productivity declines over time, leading to flock disposal for economic reasons at 72–80 weeks of age. Identifying and removing low-producing hens can enhance economic sustainability by maximising the performance of high-performing hens and conserving feed resources. Additionally, more space per productive hen can improve overall welfare. Current methods for identifying low-producing hens are subjective and challenging, particularly in large operations. Thus, objective culling techniques are needed. This study evaluated hen productivity by examining physiological and thermal changes. We correlated body colour and temperature with productivity criteria such as egg production and the feed conversion ratio (FCR). Twelve white Lohmann LSL and 12 Lohmann Brown hens of 83–100 weeks of age were included in the trial, which was conducted over an 18-week period. Hens with an egg production rate below 60% or FCR above 3 were deemed low-producing. Weekly thermal imaging captured head and foot temperatures, while spectrophotometry and digital imaging recorded the colour of the feet, combs, and beaks. A significant correlation between productivity and RGB values was found in both hybrid lines. These findings suggest the potential practical application of these techniques in poultry houses, especially as the available technological advances.

**Keywords:** culling, LAB colour space, laying hen, skin colour, spectrophotometer, thermal camera

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### Introduction

Advancements in genetics and poultry farming have led to the utilisation of hybrid laying hens that can lay over 300 eggs annually, to meet the escalating demand for animal protein (Pelletier *et al.*, 2018; Sarica *et al.*, 2018; Özentürk & Yıldız, 2021). These laying hybrids commence egg laying at 18–20 weeks of age and reach peak productivity at 23–25 weeks of age. However, productivity gradually declines, reaching approximately 70%–75% at 72 weeks of age and 65% at 80 weeks of age. Despite a 60% or higher egg production rate during this phase, economic reasons prompt flock disposal (Jacob *et al.*, 2014; Bain *et al.*, 2016; Gautron *et al.*, 2021; Eltahan *et al.*, 2023; Hy-Line, 2024), often directing these birds towards meat production (Gautron *et al.*, 2021; Sabikun *et al.*, 2021; Fan & Wu, 2022).

Ensuring economic sustainability in poultry farming necessitates the removal of unproductive birds from the poultry house (Altahat *et al.*, 2012; Alilo, 2017; Yusuf & Lacin, 2020). Practical culling involves assessing each hen at the end of the production year and removing those with low vitality, a

weaker disposition, and clear signs of low productivity (Alilo, 2017). Eliminating low-producing hens ensures several advantages, including reduced feed consumption, decreased stress from overcrowding by creating more space for high-performing hens, minimised disease occurrences, and extended productivity of efficient hens (Alilo, 2017; Yusuf & Lacin, 2020). Extending the laying period of high-yielding hens reduces the need for replacement birds and has potential positive impacts on resource utilisation, providing sustainability, economic, and welfare benefits (Preisinger, 2018; Fernyhough *et al.*, 2020). Environmental assessments have highlighted that flock renewal contributes significantly to the harmful environmental effects of intensive egg production, with only hen feed production making a larger contribution (Abín *et al.*, 2018). Studies suggest that extending the lifespan of laying hens reduces the demand for resources such as pullets and associated rearing resources, promotes more efficient use of diminishing resources such as soil, water, and feed raw materials, and leads to waste reduction and a reduced carbon footprint (Bain *et al.*, 2016). Another study demonstrated that a reduction in the use of new bird resources and a 10-week increase in the use of existing birds can conserve approximately 1 g of potentially polluting nitrogen per dozen eggs produced (Molnár *et al.*, 2016). Longer laying cycles per egg may thus reduce resource use and provide environmental benefits (Bain *et al.*, 2016; Traore & Doyon, 2023).

The welfare advantages of culling unproductive hens from the flock and utilising high-yielding hens for extended periods might be especially apparent in markets where moulting is a standard practice. If a longer egg-laying cycle is economically comparable to a typical cycle involving moulting, the economic rationale for moulting, which causes a 25% body weight loss and has about a 20% mortality rate, could consequently become obsolete (Sariozkan *et al.*, 2016; Fernyhough *et al.*, 2020; Gautron *et al.*, 2021). Additionally, egg weights tend to increase with age (Bain *et al.*, 2016; Molnár *et al.*, 2016; Tůmová *et al.*, 2017; Simeon *et al.*, 2018; Özentürk & Yıldız, 2020), contributing positively to the value chain. However, despite an egg production rate of approximately 60% or more at 72–85 weeks of age in commercial poultry farming, distinguishing productive and low-producing birds remains challenging because of large operating capacities.

In small-scale and hobbyist breeding, breeders conduct selective culling for efficiency by visually assessing factors such as vitality, health, feather quality, temperament, activity, and skin discolouration of specific body parts, such as the comb, eye ring, beak, cloaca, and feet (Claybaugh, 1947; Kekeocha, 1985; Oleforuh-Okoleh, 2011; Alilo, 2017). Observations suggest that highly productive laying hens typically exhibit large, bright-red, and glossy combs and wattles, while less productive hens tend to have smaller, dull, and shrivelled combs and wattles. Additionally, there is a distinction in beak colour, with productive laying hens usually having a bleached beak, whereas less efficient layers often have a yellow beak. It has been noted that hens with poor egg production rates may display rounded, yellow, or progressively yellowing feet and shanks, while high-yielding hens typically have bleached and triangular shanks (Claybaugh, 1947; Kekeocha, 1985; Haile-Mariam, 1995; Page, 2006; Alilo, 2017). However, these observations are subjective, and are contingent upon the breeder's experience (Page, 2006; Alilo, 2017; Yusuf & Lacin, 2020). Moreover, highly productive hens tend to be more active and nervous than lower-yielding layers (Alilo, 2017), and have higher body temperatures because of their increased metabolic rates, whereas unproductive and broody hens generally exhibit relatively lower body temperatures (Sarica *et al.*, 2018; Yusuf & Lacin, 2020).

Objective methodologies rely on data and facilitate standardised applications. Recent studies have emphasised the need to integrate technological tools within farms, to diminish human involvement in poultry management and establish data-driven decision-making processes (Ren *et al.*, 2020; Patel *et al.*, 2022). In the realm of biotechnological studies, systems equipped with environmental monitoring capabilities – such as robots, artificial intelligence, and computerised learning – offer credible means for herd management. These tools enable the assessment of environmental conditions and the identification of sick birds within hen houses (Usher *et al.*, 2017; Özentürk *et al.*, 2023).

The overarching objective within animal production systems is to enhance sustainability by minimising the environmental impact. This involves improving the feed conversion ratio (FCR), mitigating production losses caused by environmental stressors, and extending the productive lifespan of animals (Hume *et al.*, 2011; Gautron *et al.*, 2021). The aim of this study was to establish an optimal culling methodology for identifying low-producing hens among both white and brown hybrid laying hens. Its primary goals were to remove low-performing hens from the flock and improve economic sustainability by maximising the utilisation of productive genetic material. Utilising a digital camera and spectrophotometer, the study assessed colour changes in the hens' combs, beaks, and feet, and

measured body temperature with a thermal camera. In this study, both colour change and temperature were associated with productivity, indicating that these objective techniques offer an innovative method for identifying and culling low-performing hens. This study therefore has the potential to contribute to the development of technological systems for discerning productive and unproductive hens through objective methods.

## Materials and methods

The research was conducted at the Poultry Trial Unit of the Atatürk University Food and Livestock Application and Research Center, and was ethically approved by the Atatürk University Faculty of Veterinary Medicine Unit Ethics Committee (protocol no: 2021/1, dated 18/01/2021).

This investigation utilised 12 Lohmann Brown and 12 Lohmann LSL hybrid laying hens, totalling 24 hens. Hens with approximately 70% egg production at 82 weeks of age, reared in commercial production cages, were individually transferred to the trial unit. To ensure uniformity, their body weights were measured, and they were placed individually in cages within the trial unit. Each cage had a settlement area of 1350 cm<sup>2</sup> and was equipped with separate feeding areas and nipple drinkers for each hen. The trial commenced one week after the hens were relocated to the new cages and lasted a total of 18 weeks, during which the hens were 83–100 weeks of age. The experimental unit housing the hens lacked windows and was maintained at 16–24 °C using ventilation and heating system sensors. Illumination was provided by fluorescent lamps (4000 Kelvin) emitting white light for 16 hours daily.

Throughout the trial, the hens had access to water and feed *ad libitum*. The feed provided contained 2720 Kcal metabolisable energy/kg and 15.65% crude protein (Table 1), and was procured from a commercial feed mill.

**Table 1** The ingredients and nutritional composition of the feed provided to the laying hens

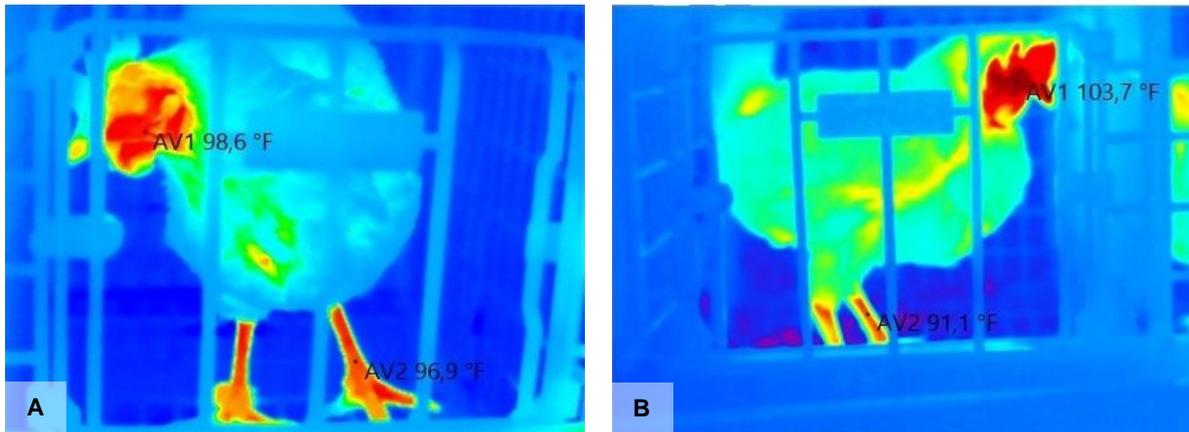
| Ingredients              | %     | Nutritional composition                   |       |
|--------------------------|-------|---|-------|
| Wheat                    | 15.00 | <b>Metabolisable energy (Kcal/kg)</b>     | 2720  |
| Maize                    | 52.08 | <b>Crude protein (%)</b>                  | 15.65 |
| Soyabean meal            | 14.92 | <b>Calcium (%)</b>                        | 3.83  |
| Sunflower seed meal      | 4.93  | <b>Phosphorus (%)</b>                     | 0.41  |
| Limestone                | 9.25  | <b>Digestible phosphorus (%)</b>          | 0.29  |
| Dicalcium phosphate      | 1.35  | <b>Sodium (%)</b>                         | 0.15  |
| Vegetable oil            | 1.59  | <b>Chloride (%)</b>                       | 0.15  |
| DL-methionine            | 0     | <b>Lysine (%)</b>                         | 0.70  |
| L-lysine                 | 0.02  | <b>Digestible lysine (%)</b>              | 0.57  |
| Enzyme                   | 0.27  | <b>Methionine (%)</b>                     | 0.33  |
| Sodium bicarbonate       | 0.16  | <b>Digestible methionine (%)</b>          | 0.27  |
| Salt                     | 0.19  | <b>Methionine/cysteine (%)</b>            | 0.61  |
| Vitamin mineral premixes | 0.25  | <b>Digestible methionine/cysteine (%)</b> | 0.50  |
|                          |       | <b>Tryptophan (%)</b>                     | 0.17  |
|                          |       | <b>Digestible tryptophan (%)</b>          | 0.14  |
|                          |       | <b>Threonine (%)</b>                      | 0.52  |
|                          |       | <b>Digestible threonine (%)</b>           | 0.42  |
|                          |       | <b>Linoleic acid (mg)</b>                 | 1.13  |

The daily egg production for each hen was recorded, and weekly egg production was calculated. Daily feed intake per bird was also measured using a scale accurate to 0.5 g, and the weekly average feed consumption was calculated. The FCR, which indicates the hen's ability to convert feed into eggs, was determined by dividing the consumed feed by the number of eggs produced, normalised using the average egg weight.

The formula used to calculate the FCR was:

$$FCR = \frac{\text{Feed consumption}}{\text{Egg production} \times \text{average egg weight}}$$

Hen body temperatures were measured weekly by capturing images of their head and foot areas using a thermal camera (Testo 855-2®) at a distance of 1 m (Figure 1). These thermal images were taken at 13:00 on a selected day of the week during the experiment. The camera used had a sensitivity of <30 mK at 30 °C (Yusuf & Lacin, 2020).



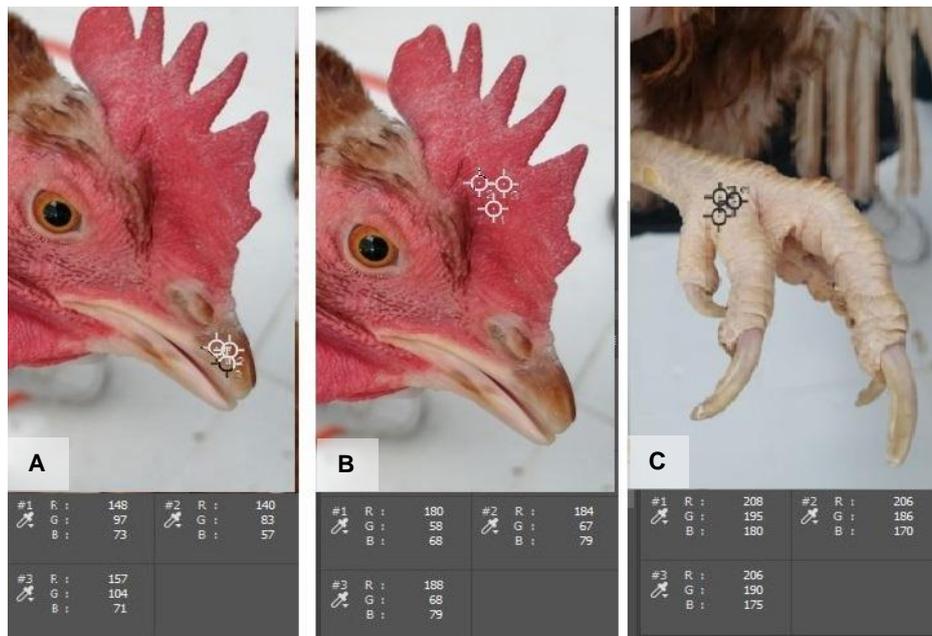
**Figure 1** Determination of the body temperatures of (A) Lohmann Brown and (B) Lohmann LSL hybrid laying hens using a thermal camera.

Colour measurements were conducted weekly by capturing images of the hens' feet, combs, and beaks. The assessment utilised a CIE Lab\* model spectrophotometer (Minolta2 Chromameter CR-300®), along with a digital camera for imaging purposes. The CIELAB, or CIE L\* a\* b\* colour system, delineates colour quantitatively across three axes (Ly *et al.*, 2020): L\*, a\*, and b\*. The L\* value indicates lightness, positioned on a vertical axis from 0 (black) to 100 (white) on the colour space diagram. The a\* value, which ranges from -128 to 127, signifies the red-green component of a colour, with positive a\* and negative a\* values indicating the red and green ends of the spectrum, respectively. The b\* value, which also ranges from -128 to 127, represents the yellow-blue component of a colour, with positive b\* and negative b\* values indicating the yellow and blue ends of the spectrum, respectively. The neutral or achromatic point resides at the centre of the plane, and the distance from the central axis is indicated by the chroma (C\*) value, which reflects the colour saturation level. The angle on the chromaticity axes is indicated by the hue (h<sub>o</sub>) value. The L\*, a\*, and b\* values can be translated into dermatological parameters, with L\* correlating with skin pigmentation levels, a\* correlating with erythema, and b\* correlating with pigmentation and tanning. The Commission Internationale de l'Éclairage (International Commission on Illumination), standardises these colour metrics. During measurements, the probe part of the device was briefly placed in contact with the targeted area for 1–2 seconds, and readings were taken.

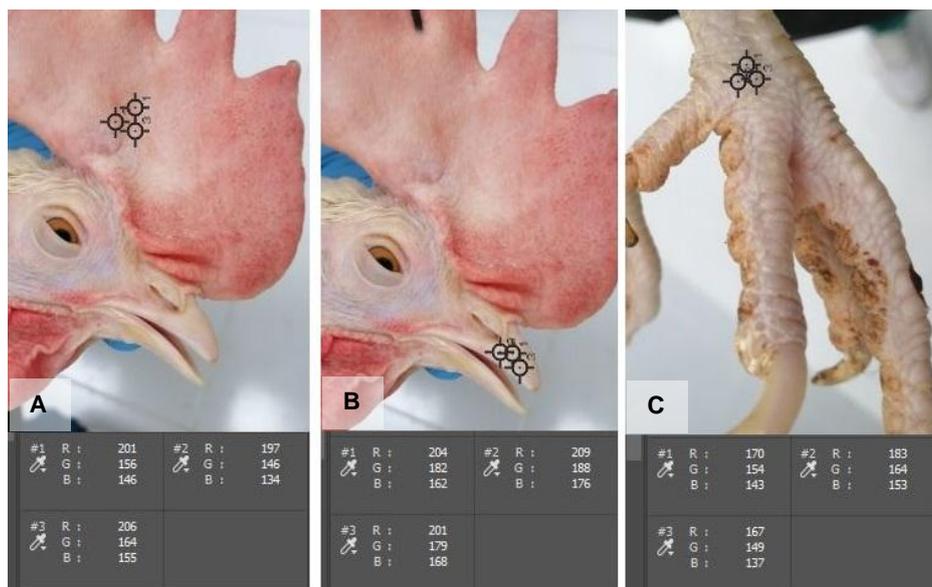
For the digital photography, each bird was removed from its cage and photographed from a distance of 30 cm. RGB colour codes were determined using Adobe Photoshop® (v. 24.1.0.) to analyse the images captured by the digital camera (Calvini *et al.*, 2020; Annum & Poku, 2021). Within Adobe Photoshop®, the means of the red, green, and blue colour scores were obtained by selecting three areas, each 100 × 100 pixels in size, from the feet, beaks, and combs of each bird (Figures 2 and 3). RGB values ranged from 0 to 255 (Kanjanasoontara & Suppitaksakul, 2023; Leyferman *et al.*, 2023).

The effects of hybrid type and week on body temperature and colour values were examined using the general linear model procedure, and Duncan's multiple comparison was used for the multiple comparison tests. Hens with an egg production rate below 60% or FCR above 3 were recorded as having low productivity. Descriptive analysis was performed to determine the average values of the measured parameters for hens with low (<60%) and high (>60%) egg production and low (FCR >3) and

high ( $FCR \leq 3$ ) feed efficiencies. Receiver operating characteristic (ROC) curves were employed to distinguish between high ( $>60\%$ ) and low ( $<60\%$ ) egg production, represented as 1 and 0, and to categorise those with low ( $FCR > 3$ ) and high ( $FCR \leq 3$ ) feed efficiencies. In this model, body temperature, which was considered a potential indicator for determining egg production, spectrophotometer colour values ( $L^*$ ,  $a^*$ , and  $b^*$ ), and the correlation between relevant body parts and RGB pixel values obtained from digital camera images, were evaluated. The parameters associated with the area under the ROC curve (AUC) were compared to select the most descriptive indicator and its cut-off point values. The outcomes in this area were quantified using the AUC, along with the 95% confidence interval. All statistical analyses were conducted using the SPSS software package (version 20.0).



**Figure 2** Determination of the colours of the (A) beaks, (B) combs, and (C) feet of Lohmann Brown hybrid hens, as indicated by RGB values.



**Figure 3** Determination of the colours of the (A) combs, (B) beaks, and (C) feet of Lohmann LSL hybrid hens, as indicated by RGB values.

## Results and discussion

Egg production and FCR data obtained during the trial are detailed in Table 2. Over the period of 83–100 weeks, average egg production rates were recorded as 79.96% for the Lohmann LSL and 76.85% for the Lohmann Brown hens ( $P > 0.05$ ). The impact of age on egg production and FCR was found to be statistically significant for both hybrids.

**Table 2** Weekly egg production and feed conversion ratios for hybrid laying hens

| Age (weeks)      | Egg production (%) |               | Feed conversion ratio |               |
|------------------|--------------------|---------------|-----------------------|---------------|
|                  | Lohmann LSL        | Lohmann Brown | Lohmann LSL           | Lohmann Brown |
| 83               | 83.33              | 79.76         | 2.52                  | 2.47          |
| 84               | 78.57              | 77.38         | 2.59                  | 2.89          |
| 85               | 86.90              | 83.33         | 2.12                  | 2.59          |
| 86               | 83.33              | 82.14         | 2.39                  | 2.42          |
| 87               | 89.29              | 90.48         | 2.04                  | 2.11          |
| 88               | 85.71              | 85.71         | 2.25                  | 2.95          |
| 89               | 90.48              | 89.29         | 2.16                  | 2.10          |
| 90               | 88.10              | 88.10         | 2.14                  | 2.37          |
| 91               | 88.10              | 80.95         | 2.20                  | 2.57          |
| 92               | 86.90              | 80.95         | 2.33                  | 3.32          |
| 93               | 90.48              | 84.52         | 2.26                  | 2.40          |
| 94               | 86.91              | 73.81         | 2.48                  | 3.17          |
| 95               | 78.57              | 73.81         | 2.90                  | 3.30          |
| 96               | 76.19              | 73.81         | 3.11                  | 3.06          |
| 97               | 61.90              | 69.05         | 3.69                  | 3.60          |
| 98               | 64.29              | 60.71         | 4.09                  | 4.01          |
| 99               | 63.09              | 50.00         | 3.69                  | 5.43          |
| 100              | 57.14              | 59.52         | 4.33                  | 4.26          |
| SEM              | 7.369              | 7.369         | 0.613                 | 0.613         |
| Mean ± SE        | 79.96 ± 1.74       | 76.85 ± 1.74  | 2.74 ± 0.14           | 3.06 ± 0.14   |
| <b>P-values:</b> |                    |               |                       |               |
| Hybrid           |                    | 0.206         |                       | 0.121         |
| Age (weeks)      |                    | <0.001        |                       | <0.001        |
| Hybrid x Age     |                    | 1.000         |                       | 0.997         |

SEM: standard error of the mean, SE: standard error

The study concluded that there was an increase in egg production for both genotypes until the 93rd week of age, with the Lohmann LSL hens peaking at 90.48% and the Lohmann Brown hens peaking at 84.52%, and a decrease from the 94th week onwards (Table 2). In contrast, it was reported that the average egg production of these hens before the experiment was only approximately 70%. However, prior to the trial, the hens were housed in a commercial poultry house, with six birds per cage and 625 cm<sup>2</sup> per bird, whereas during the trial, from 82 weeks of age onwards, they were housed in new cages with separate compartments and 1350 cm<sup>2</sup> per bird. The observed increase in egg production was thus linked to a positive impact of the lower stocking density used during the trial (Erensoy *et al.*, 2021; Özentürk & Yıldız, 2021; Hanh *et al.*, 2023; Wan *et al.*, 2023). This suggests that a reduced stocking density provides a more comfortable environment for hens, potentially influencing their productivity. This outcome calls for further scientific exploration into the positive effects of reducing stocking density on laying hens during the latter phase of egg production (Yusuf & Lacin, 2020). Moreover, this finding supports the suggestion that reducing the stocking density by culling unproductive

hens may mitigate negative effects and prolong the utilisation of high-producing birds, aligning with the research objectives.

The FCR, another productivity metric, was calculated to be 2.74 for the Lohmann LSL hens and 3.06 for the Lohmann Brown hens ( $P > 0.05$ ). The FCR values initially decreased, but later increased. This observed change in the FCR over time correlates with the fluctuations in egg production.

**Table 3** Receiver operating characteristic analysis for the egg production and feed conversion ratios of Lohmann LSL hybrid laying hens

| Test result variables      | Egg production |            |         |                           |             | Feed conversion ratio |            |         |                           |             |
|----------------------------|----------------|------------|---------|---------------------------|-------------|-----------------------|------------|---------|---------------------------|-------------|
|                            | AUC            | Std. error | P-value | Confidence interval (95%) |             | AUC                   | Std. error | P-value | Confidence interval (95%) |             |
|                            |                |            |         | Lower bound               | Upper bound |                       |            |         | Lower bound               | Upper bound |
| <b>Digital camera</b>      |                |            |         |                           |             |                       |            |         |                           |             |
| Comb red                   | 0.580          | 0.051      | 0.115   | 0.479                     | 0.681       | 0.565                 | 0.052      | 0.197   | 0.464                     | 0.666       |
| Comb green                 | 0.498          | 0.051      | 0.974   | 0.399                     | 0.598       | 0.470                 | 0.050      | 0.546   | 0.372                     | 0.567       |
| Comb blue                  | 0.509          | 0.051      | 0.860   | 0.410                     | 0.608       | 0.482                 | 0.050      | 0.726   | 0.384                     | 0.581       |
| Beak red                   | 0.594          | 0.051      | 0.064   | 0.495                     | 0.694       | 0.589                 | 0.050      | 0.079   | 0.491                     | 0.687       |
| Beak green                 | 0.575          | 0.053      | 0.142   | 0.472                     | 0.678       | 0.573                 | 0.052      | 0.150   | 0.472                     | 0.674       |
| Beak blue                  | 0.562          | 0.055      | 0.222   | 0.455                     | 0.669       | 0.555                 | 0.054      | 0.278   | 0.449                     | 0.661       |
| Foot red                   | 0.669          | 0.045      | 0.001   | 0.581                     | 0.757       | 0.676                 | 0.044      | <0.001  | 0.589                     | 0.763       |
| Foot green                 | 0.677          | 0.045      | <0.001  | 0.590                     | 0.765       | 0.685                 | 0.044      | <0.001  | 0.598                     | 0.772       |
| Foot blue                  | 0.690          | 0.044      | <0.001  | 0.603                     | 0.777       | 0.694                 | 0.044      | <0.001  | 0.608                     | 0.780       |
| <b>Spectrophotometer</b>   |                |            |         |                           |             |                       |            |         |                           |             |
| Comb L*                    | 0.527          | 0.046      | 0.590   | 0.438                     | 0.617       | 0.498                 | 0.044      | 0.962   | 0.411                     | 0.585       |
| Comb a*                    | 0.451          | 0.048      | 0.332   | 0.356                     | 0.545       | 0.482                 | 0.047      | 0.726   | 0.389                     | 0.575       |
| Comb b*                    | 0.386          | 0.048      | 0.026   | 0.293                     | 0.480       | 0.392                 | 0.047      | 0.031   | 0.299                     | 0.484       |
| Comb chroma                | 0.424          | 0.049      | 0.138   | 0.329                     | 0.520       | 0.451                 | 0.049      | 0.327   | 0.355                     | 0.546       |
| Comb hue                   | 0.504          | 0.048      | 0.942   | 0.409                     | 0.599       | 0.472                 | 0.047      | 0.580   | 0.380                     | 0.564       |
| Beak L*                    | 0.481          | 0.052      | 0.708   | 0.379                     | 0.583       | 0.481                 | 0.052      | 0.709   | 0.379                     | 0.584       |
| Beak a*                    | 0.423          | 0.050      | 0.131   | 0.325                     | 0.521       | 0.420                 | 0.049      | 0.113   | 0.323                     | 0.517       |
| Beak b*                    | 0.491          | 0.052      | 0.860   | 0.389                     | 0.593       | 0.481                 | 0.051      | 0.701   | 0.380                     | 0.581       |
| Beak chroma                | 0.467          | 0.052      | 0.512   | 0.364                     | 0.569       | 0.454                 | 0.052      | 0.360   | 0.353                     | 0.555       |
| Beak hue                   | 0.564          | 0.050      | 0.208   | 0.467                     | 0.661       | 0.560                 | 0.049      | 0.233   | 0.464                     | 0.656       |
| Foot L*                    | 0.350          | 0.049      | 0.003   | 0.253                     | 0.446       | 0.354                 | 0.049      | 0.004   | 0.259                     | 0.450       |
| Foot a*                    | 0.623          | 0.054      | 0.015   | 0.518                     | 0.729       | 0.645                 | 0.051      | 0.004   | 0.545                     | 0.745       |
| Foot b*                    | 0.412          | 0.055      | 0.085   | 0.305                     | 0.519       | 0.447                 | 0.056      | 0.294   | 0.338                     | 0.556       |
| Foot chroma                | 0.509          | 0.058      | 0.856   | 0.395                     | 0.624       | 0.541                 | 0.057      | 0.421   | 0.429                     | 0.653       |
| Foot hue                   | 0.511          | 0.058      | 0.826   | 0.397                     | 0.626       | 0.487                 | 0.057      | 0.791   | 0.374                     | 0.599       |
| <b>Thermal camera (°F)</b> |                |            |         |                           |             |                       |            |         |                           |             |
| Head temp.                 | 0.363          | 0.050      | 0.007   | 0.265                     | 0.462       | 0.350                 | 0.047      | 0.003   | 0.257                     | 0.442       |
| Foot temp.                 | 0.475          | 0.053      | 0.623   | 0.371                     | 0.579       | 0.483                 | 0.053      | 0.740   | 0.380                     | 0.587       |

AUC: area under the curve, Std.: standard, temp.: temperature. L\*: represents darkness to lightness, a\*: represents greenness to redness, and b\*: represents blueness to yellowness.

In this study, we investigated body colour changes as the initial physiological markers for identifying low-producing hens. Receiver operating characteristic analysis was conducted to discern the potential link between foot, comb, and beak colour values and egg production (low: <60% and

high: >60%) and feed efficiency (low: FCR >3 and high: FCR ≤3). This analysis aimed to establish optimal thresholds for these parameters and classify hens as low- or high-producing based on these values. The parameters associated with egg production and FCR exhibited similar trends (Tables 3 and 4).

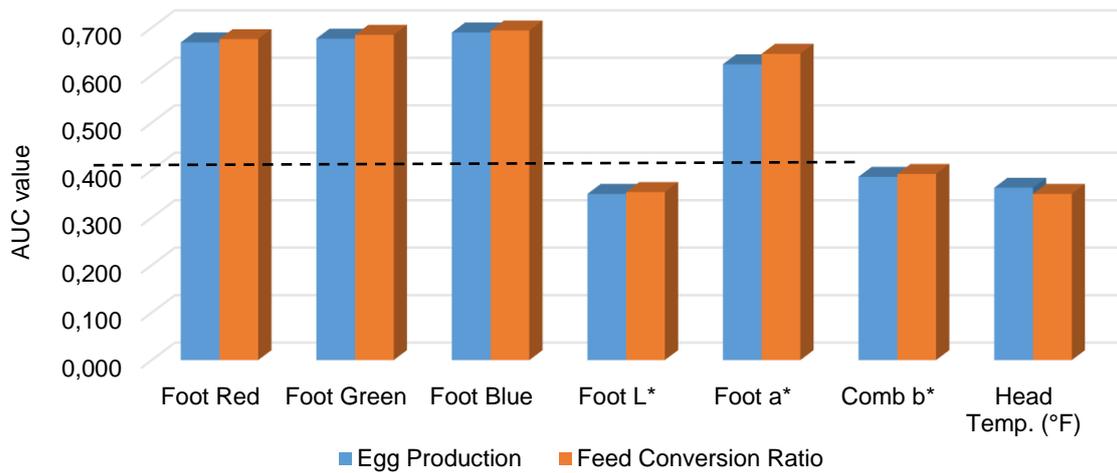
**Table 4** Receiver operating characteristic analysis for the egg production and feed conversion ratios of Lohmann Brown hybrid laying hens

| Test result variables      | Egg production |            |         |                           |             | Feed conversion ratio |            |         |                         |             |
|----------------------------|----------------|------------|---------|---------------------------|-------------|-----------------------|------------|---------|-------------------------|-------------|
|                            | AUC            | Std. error | P-value | Confidence interval (95%) |             | AUC                   | Std. error | P-value | Confidence interval 95% |             |
|                            |                |            |         | Lower bound               | Upper bound |                       |            |         | Lower bound             | Upper bound |
| <b>Digital camera</b>      |                |            |         |                           |             |                       |            |         |                         |             |
| Comb red                   | 0.478          | 0.045      | 0.637   | 0.390                     | 0.566       | 0.470                 | 0.045      | 0.521   | 0.382                   | 0.557       |
| Comb green                 | 0.346          | 0.043      | 0.001   | 0.262                     | 0.430       | 0.351                 | 0.043      | 0.002   | 0.266                   | 0.436       |
| Comb blue                  | 0.371          | 0.044      | 0.006   | 0.285                     | 0.456       | 0.377                 | 0.044      | 0.009   | 0.291                   | 0.464       |
| Beak red                   | 0.584          | 0.044      | 0.073   | 0.497                     | 0.671       | 0.576                 | 0.045      | 0.110   | 0.487                   | 0.664       |
| Beak green                 | 0.557          | 0.048      | 0.223   | 0.463                     | 0.650       | 0.559                 | 0.048      | 0.211   | 0.464                   | 0.654       |
| Beak blue                  | 0.557          | 0.049      | 0.221   | 0.461                     | 0.653       | 0.562                 | 0.049      | 0.193   | 0.465                   | 0.658       |
| Foot red                   | 0.636          | 0.046      | <0.001  | 0.546                     | 0.726       | 0.628                 | 0.046      | 0.007   | 0.537                   | 0.718       |
| Foot green                 | 0.668          | 0.043      | <0.001  | 0.582                     | 0.753       | 0.667                 | 0.044      | <0.001  | 0.582                   | 0.752       |
| Foot blue                  | 0.695          | 0.042      | <0.001  | 0.613                     | 0.777       | 0.692                 | 0.042      | <0.001  | 0.610                   | 0.774       |
| <b>Spectrophotometer</b>   |                |            |         |                           |             |                       |            |         |                         |             |
| Comb L*                    | 0.294          | 0.043      | <0.001  | 0.210                     | 0.378       | 0.300                 | 0.045      | <0.001  | 0.213                   | 0.388       |
| Comb a*                    | 0.615          | 0.046      | 0.014   | 0.525                     | 0.705       | 0.606                 | 0.047      | 0.026   | 0.513                   | 0.698       |
| Comb b*                    | 0.350          | 0.045      | 0.001   | 0.262                     | 0.438       | 0.330                 | 0.044      | <0.001  | 0.244                   | 0.417       |
| Comb chroma                | 0.564          | 0.046      | 0.172   | 0.474                     | 0.653       | 0.550                 | 0.047      | 0.289   | 0.459                   | 0.642       |
| Comb hue                   | 0.318          | 0.050      | <0.001  | 0.221                     | 0.415       | 0.315                 | 0.051      | <0.001  | 0.216                   | 0.415       |
| Beak L*                    | 0.413          | 0.049      | 0.062   | 0.317                     | 0.509       | 0.423                 | 0.050      | 0.104   | 0.324                   | 0.522       |
| Beak a*                    | 0.517          | 0.050      | 0.722   | 0.419                     | 0.614       | 0.501                 | 0.051      | 0.977   | 0.402                   | 0.601       |
| Beak b*                    | 0.423          | 0.051      | 0.099   | 0.323                     | 0.523       | 0.418                 | 0.051      | 0.084   | 0.317                   | 0.519       |
| Beak chroma                | 0.471          | 0.053      | 0.535   | 0.367                     | 0.575       | 0.466                 | 0.054      | 0.472   | 0.361                   | 0.571       |
| Beak hue                   | 0.414          | 0.050      | 0.065   | 0.316                     | 0.512       | 0.428                 | 0.051      | 0.128   | 0.328                   | 0.528       |
| Foot L*                    | 0.475          | 0.048      | 0.595   | 0.380                     | 0.570       | 0.492                 | 0.050      | 0.867   | 0.394                   | 0.590       |
| Foot a*                    | 0.530          | 0.047      | 0.526   | 0.438                     | 0.622       | 0.514                 | 0.048      | 0.762   | 0.421                   | 0.608       |
| Foot b*                    | 0.442          | 0.047      | 0.217   | 0.351                     | 0.534       | 0.444                 | 0.047      | 0.236   | 0.351                   | 0.536       |
| Foot chroma                | 0.500          | 0.048      | 0.994   | 0.406                     | 0.593       | 0.506                 | 0.048      | 0.898   | 0.412                   | 0.600       |
| Foot hue                   | 0.494          | 0.048      | 0.899   | 0.401                     | 0.588       | 0.514                 | 0.048      | 0.764   | 0.420                   | 0.609       |
| <b>Thermal camera (°F)</b> |                |            |         |                           |             |                       |            |         |                         |             |
| Head temp.                 | 0.524          | 0.052      | 0.615   | 0.422                     | 0.625       | 0.534                 | 0.053      | 0.475   | 0.430                   | 0.638       |
| Foot temp.                 | 0.519          | 0.046      | 0.684   | 0.429                     | 0.609       | 0.530                 | 0.046      | 0.523   | 0.439                   | 0.621       |

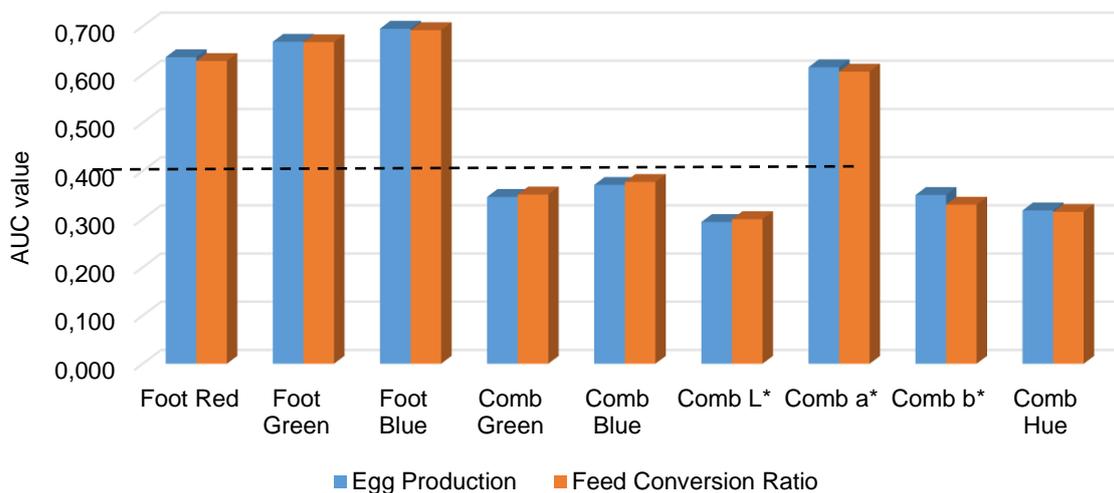
AUC: area under the curve, Std.: standard, temp.: temperature. L\*: represents darkness to lightness, a\*: represents greenness to redness, and b\*: represents blueness to yellowness.

The ROC analysis results for egg production and FCR in Lohmann LSL and Lohmann Brown hens are shown in Figures 4 and 5, respectively. To improve clarity and readability, only significant variables ( $P < 0.05$ ) have been included in these figures.

For Lohmann LSL hens, the foot colour variables (red, green, and blue) exhibited the highest AUC values for both egg production and FCR (Figure 4, Table 3). The strongest predictors were 'Foot blue' (AUC = 0.690,  $P < 0.001$ ) for egg production and 'Foot green' (AUC = 0.685,  $P < 0.001$ ) for FCR. Additionally, head temperature showed a significant relationship with both parameters, with lower AUC values indicating weaker predictive capacity. In Lohmann Brown hens, significant predictors included foot and comb colour variables (Figure 5, Table 4). 'Foot blue' (AUC = 0.695,  $P < 0.001$ ) demonstrated the strongest correlation with egg production, while 'Foot green' (AUC = 0.667,  $P < 0.001$ ) was a key predictor for the FCR. Comb parameters (L\*, a\*, b\*, and hue) also played a role, with AUC values ranging between 0.294 and 0.615, suggesting moderate predictive power.



**Figure 4** Area under the curve (AUC) values for significant predictors of egg production and feed conversion ratio in Lohmann LSL hybrid laying hens.



**Figure 5** Area under the curve (AUC) values for significant predictors of egg production and feed conversion ratio in Lohmann Brown hybrid laying hens.

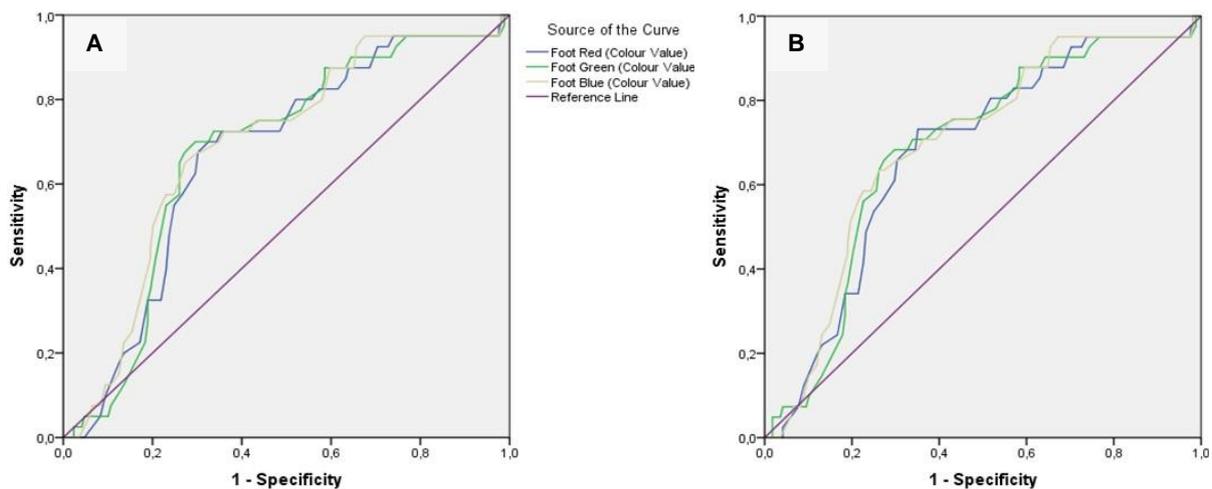
In both genetic strains, the AUC values for foot colour (red, green, and blue) were notably higher than for the other parameters. Within each hybrid group, specific cut-off points for foot colour were established (Tables 5 and 6). These cut-off values represent the optimal thresholds where sensitivity and specificity peak, indicating accuracy in selecting birds based on egg production or FCR. Our findings

revealed that the specific cut-off values for foot colour concerning egg production in the Lohmann LSL hybrid hens were 210.5 for red, 192.0 for green, and 173.5 for blue. For FCR, these values were 208.5, 192.0, and 174.5 respectively (Figure 6, Table 5).

**Table 5** Receiver operating characteristic analysis of Lohmann LSL hybrid laying hens' foot colour for egg production and feed conversion ratio

|   | AUC   | Std. error | P-value | Cut-off point | Sensitivity (TP) | 1 - Specificity (FP) |
|---|-------|------------|---------|---------------|------------------|----------------------|
| <b>Parameters for egg production</b>        |       |            |         |               |                  |                      |
| Foot DC red colour                          | 0.669 | 0.045      | 0.001   | 210.50        | 0.700            | 0.325                |
| Foot DC green colour                        | 0.677 | 0.045      | <0.001  | 192.00        | 0.700            | 0.296                |
| Foot DC blue colour                         | 0.690 | 0.044      | <0.001  | 173.50        | 0.650            | 0.272                |
| <b>Parameters for feed conversion ratio</b> |       |            |         |               |                  |                      |
| Foot DC red colour                          | 0.676 | 0.044      | <0.001  | 208.50        | 0.732            | 0.351                |
| Foot DC green colour                        | 0.685 | 0.044      | <0.001  | 192.00        | 0.683            | 0.298                |
| Foot DC blue colour                         | 0.694 | 0.044      | <0.001  | 174.50        | 0.634            | 0.262                |

AUC: area under the curve, Std.: standard, TP: true positive, FP: false positive, DC: digital camera



**Figure 6** Receiver operating characteristic curves for foot colour in Lohmann LSL hens for (A) egg production and (B) feed conversion ratio.

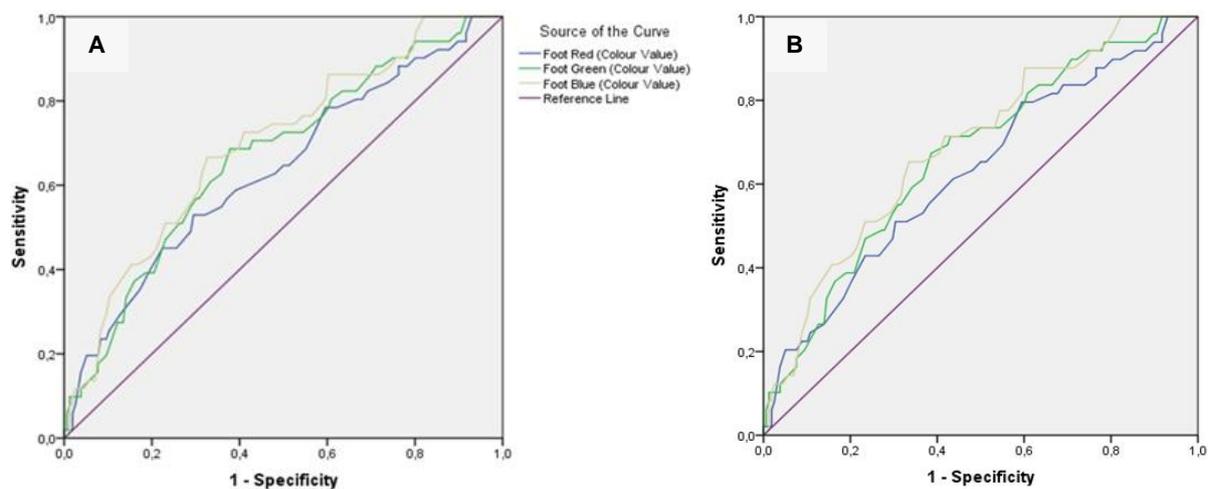
In the Lohmann Brown hybrid laying hens, consistent cut-off values were observed across both egg production and feed conversion ratio parameters: 212.0 for red, 188.5 for green, and 167.5 for blue (Figure 7, Table 6). According to the findings of this study, approximately 70% of the birds showing values higher than the cut-off points indicated for foot colour in Tables 5 and 6 may have low production potential, which reflects a statistically reliable level of discrimination (DeSalvo *et al.*, 2005; Gur *et al.*, 2010; Nezic *et al.*, 2016).

Comprehensive descriptive data regarding the average egg production and FCR of the hybrid laying hens are outlined in Table 7. To our knowledge, while previous studies have explored the relationships between various body colours and production efficiency, this research uniquely quantifies colour values. Consequently, this study is the first to discuss positive threshold values concerning the correlation between body skin colour and production.

**Table 6** Receiver operating characteristic analysis of Lohmann Brown laying hens' foot colour for egg production and feed conversion ratio

|   | AUC   | Std. error | P-value | Cut-off point | Sensitivity (TP) | 1 - Specificity (FP) |
|---|-------|------------|---------|---------------|------------------|----------------------|
| <b>Parameters for egg production</b>        |       |            |         |               |                  |                      |
| Foot DC red colour                          | 0.636 | 0.046      | 0.004   | 212.00        | 0.529            | 0.295                |
| Foot DC green colour                        | 0.668 | 0.043      | <0.001  | 188.50        | 0.686            | 0.378                |
| Foot DC blue colour                         | 0.695 | 0.042      | <0.001  | 167.50        | 0.667            | 0.327                |
| <b>Parameters for feed conversion ratio</b> |       |            |         |               |                  |                      |
| Foot DC red colour                          | 0.628 | 0.046      | 0.007   | 212.00        | 0.510            | 0.304                |
| Foot DC green colour                        | 0.667 | 0.044      | <0.001  | 188.50        | 0.673            | 0.386                |
| Foot DC blue colour                         | 0.692 | 0.042      | <0.001  | 167.50        | 0.653            | 0.335                |

AUC: area under the curve, Std.: standard, TP: true positive, FP: false positive, DC: digital camera



**Figure 7** Receiver operating characteristic curves for foot colour in Lohmann Brown hens for (A) egg production and (B) feed conversion ratio.

**Biological mechanisms underlying productivity indicators**

The physiological basis for these correlations is linked to pigment metabolism and thermoregulation in laying hens. The changes in colour observed across the various body parts of the hens reveal underlying pigmentation mechanisms (Singh *et al.*, 2022). Pigmentation in non-feathered tissues, such as the skin and shanks, involves carotenoids and melanin, with carotenoids yielding yellow hues and melanin contributing to black colouring (Gowda *et al.*, 2020). Notably, xanthophyll carotenoids play a unique role in poultry, influencing both skin colour in laying hens and egg yolk colouration. As hens reach sexual maturity, xanthophyll supplies in the muscles and skin shift to the ovaries, and are eventually excreted into the egg yolks (Heying *et al.*, 2014). During egg production, hens draw xanthophyll carotenoids from their skin, affecting skin colour as the stored carotenoids decrease over time (Shevchenko *et al.*, 2021; Alvarado *et al.*, 2023; Belwal *et al.*, 2023). This process, which intensifies with prolonged laying, serves as a visible biomarker of sustained egg production. In breeds with yellow skin, the pigment responsible for yolk colouration also tints the vents, eye rings, beaks, skin, and feet. However, with the onset of laying, this yellow pigment gradually diminishes (Page, 2006; Alilo, 2017). Similarly, variations in comb and wattle colouration are influenced by oestrogen levels, which regulate blood circulation and metabolic activity (Dong *et al.*, 2019). Hens exhibiting more vibrant comb colours typically have higher oestrogenic activity, supporting sustained egg production.

**Table 7** Average values of foot colour parameters for egg production and feed conversion ratio (mean  $\pm$  standard error) in hybrid laying hens

| Parameter                    |              | Lohmann LSL       | Lohmann Brown     |
|------------------------------|--------------|-------------------|-------------------|
| <b>Egg production</b>        |              |                   |                   |
| Foot red                     | Low (<60%)   | 210.48 $\pm$ 2.14 | 211.35 $\pm$ 2.07 |
|                              | High (>60%)  | 201.23 $\pm$ 1.28 | 203.81 $\pm$ 1.24 |
| Foot green                   | Low (<60%)   | 192.33 $\pm$ 2.26 | 192.86 $\pm$ 2.05 |
|                              | High (>60%)  | 182.96 $\pm$ 1.31 | 182.94 $\pm$ 1.36 |
| Foot blue                    | Low (<60%)   | 174.13 $\pm$ 2.34 | 171.59 $\pm$ 2.12 |
|                              | High (>60%)  | 163.40 $\pm$ 1.34 | 159.11 $\pm$ 1.47 |
| <b>Feed conversion ratio</b> |              |                   |                   |
| Foot red                     | FCR >3       | 210.73 $\pm$ 2.12 | 211.08 $\pm$ 2.10 |
|                              | FCR $\leq$ 3 | 201.11 $\pm$ 1.28 | 203.99 $\pm$ 1.24 |
| Foot green                   | FCR >3       | 192.68 $\pm$ 2.27 | 192.94 $\pm$ 2.07 |
|                              | FCR $\leq$ 3 | 182.82 $\pm$ 1.30 | 183.04 $\pm$ 1.35 |
| Foot blue                    | FCR >3       | 174.29 $\pm$ 2.31 | 171.65 $\pm$ 2.13 |
|                              | FCR $\leq$ 3 | 163.30 $\pm$ 1.34 | 159.25 $\pm$ 1.47 |

Our results strongly suggest that foot-derived RGB values serve as highly sensitive predictors for both genotypes. Foot colour is determined by pigments present in both the upper (epidermis) and lower (dermis) layers of the skin. The melanin content determines the darkness of the feet, while differing melanin concentrations in the epidermis and dermis produce distinctive colours like slate blue or willow green (Guni & Katule, 2013; Yu *et al.*, 2017). Furthermore, the variation in shank colour may be attributed to combinations of pigment-controlling genes responsible for colour determination. For instance, Petrus (2011) indicated that carotenoid production, dermal melanin, and epidermal melanin are regulated by genes such as *W+* and *w*, *Id* and *id+*, and *E* and *e+*, respectively. These genetic interactions result in a spectrum of shank colour shades (Petrus, 2011). Yu *et al.* (2017) reported that the tyrosinase (TYR) gene plays an important role in hen pigmentation, accounting for differences in skin colour due to TYR regulation. In other research, comb colour has been found to correlate with egg production, driven by genes like *EDN3* and *BMP7* on chromosome 20, indicating melanogenesis and folliculogenesis as significant targets for selection in high-producing hens (Dong *et al.*, 2019).

Changes in body part colouration are related to stored pigment quantity, feed type, body weight, egg size, and production duration (Page, 2006; Alilo, 2017). Reports suggest that body colour changes initially occur in the cloaca region during egg production, followed by changes in other body parts, in the sequence: eye ring, earlobe, beak, then feet (Page, 2006; Alilo, 2017). Foot colour changes only gradually, indicating a longer production period and typically taking about six months to fully manifest, depending on breed, egg size, feed type, and production rate (Guni & Katule, 2013). The yellow colour fades in the shanks' front scales first, and later in the rear scales, with the shank heels and toe tops being the last to bleach, often reflecting the bird's natural yellow colour depth (Page, 2006; Alilo, 2017). Our study's findings align with the documented timeframes for colour changes in body parts towards the end of the production period.

The analysis aimed at correlating production performance with thermal variations in the animal body revealed a notable finding: low-producing Lohmann LSL hybrid hens exhibited lower head temperature values than high-producing Lohmann LSL hens ( $P < 0.01$  for egg production,  $P < 0.01$  for FCR). However, the explanatory power of this parameter remained relatively low (AUC: 0.363 for egg production, 0.350 for FCR) (Table 3). High-producing hens exhibit greater metabolic heat production because of their sustained ovulatory cycles, increasing their peripheral blood flow and surface temperature (Brummermann & Reinertsen, 1992). Existing literature on the relationship between body temperature and production efficiency is limited. Yusuf & Lacin (2020) suggested that morning head

temperatures might serve as a more effective metric for assessing the body temperature-egg production nexus. These variations in temperature can be attributed to the lower metabolic rates of low-yielding hens such as brooder hens, during periods when eggs are not produced (Brummermann & Reinertsen, 1992). These biological mechanisms provide insight into how physiological changes can serve as objective markers for productivity assessment. Conversely, the relationship between head temperature and production was not statistically significant in the Lohmann Brown hens, indicating a genetic component in this relationship. It has been reported that activity and metabolic rate in hens may vary genetically (Ghayas *et al.*, 2020; Mattioli *et al.*, 2021). In particular, white laying hybrids originating from axe-crested white Leghorns are more mobile than brown laying hybrids (Özentürk & Yıldız, 2021). The results can thus be interpreted as indicating that hens from different hybrid lines have different metabolic rates, and this may affect the changes in body temperature in relation to egg production.

In contrast, foot temperature values in both hybrids did not exhibit a significant relationship with productivity, likely because of the insulative properties of the avian leg vasculature, which allows thermoregulation independent of metabolic heat production. This finding aligns with previous assertions that foot temperature might not serve as an appropriate criterion for identifying inefficient hens (Yusuf & Lacin, 2020). The wider standard deviation in foot temperatures decreases the precision in establishing the link between body temperature and egg production using foot temperature values. Moreover, the higher heat loss by conduction from hen feet, compounded by the potential influence of poultry house temperature and cage conditions on foot temperature, might introduce confounding variables that may affect the relationship between foot temperature and egg production.

### **Challenges and future directions**

Despite the promising results, implementing objective identification methods in large-scale poultry operations presents several challenges. Environmental factors such as lighting conditions, house temperature, and feed composition may influence skin colour and thermal readings, necessitating standardised protocols to ensure consistency. Additionally, technological limitations, including the need for automated image capture and data processing systems, must be addressed to enable real-time monitoring in commercial settings. The integration of machine-learning algorithms for automated hen classification based on colour and temperature profiles could significantly enhance the feasibility and scalability of these methods (Ren *et al.*, 2020; Özentürk *et al.*, 2024). In commercial poultry houses, establishing a standardised environment for imaging may be necessary. In free-range rearing systems, standardisation can be achieved through controlled lighting, and images can be obtained using strategically placed cameras in pop holes.

Another practical concern is the potential stress induced by frequent handling for imaging and spectrophotometry. Unlike traditional culling decisions based on subjective visual assessments, our proposed approach requires precise data collection, which may necessitate modifications in management practices to minimise flock disruption. Implementing these technologies would also require comprehensive training for farm personnel and continuous refinement of automated systems to ensure efficiency and reliability. Additionally, the removal of low-yielding birds from the flock could cause stress among the remaining birds, potentially impacting their welfare and productivity. To manage this, a phased culling approach can be implemented to reduce abrupt changes in flock dynamics. Maintaining consistent group sizes, ensuring adequate space, and minimising handling stress during removals could further help reduce disruptions.

Further research should incorporate larger sample sizes and diverse production environments to enhance the robustness of these methods. The present study was conducted under controlled conditions. However, factors such as dust accumulation on the feet, moulting periods, and ambient temperature fluctuations could potentially influence the accuracy of colour-based assessments on commercial poultry farms. Future studies should evaluate the impact of these variables on the reliability of foot colour and thermal markers on large-scale farms.

Finally, while ROC analysis provided statistically significant thresholds for distinguishing productive from unproductive hens, additional validation studies are necessary to determine the consistency of these cut-off values across different production cycles, housing systems, and genetic lines. Strengthening these methodologies through rigorous testing and refinement will be crucial for their successful integration into commercial poultry farming practices.

## Conclusions

Eliminating low-producing hens from the flock not only enhances the sustainability of egg production by saving feed resources but also extends the utilisation period of productive hens. Moreover, providing increased space per productive hen improves animal welfare, meeting the expectations of egg consumers. Our findings indicate that employing objective methods for identifying low-producing hens is feasible, with both white and brown hybrid laying hens being effectively sorted using foot RGB values towards the end of the production period. These results emphasise the potential of digital and spectrophotometric colour assessments as early indicators of productivity in laying hens. Integrating this sorting method into technological tools holds significant potential for enhancing environmental sustainability in poultry farming and prolonging the utilisation of productive animal material. However, while sorting may offer benefits in commercial poultry operations by maximising the use of productive birds, it is crucial to acknowledge that these methods are still nascent, marginal, and largely untested in larger enterprises. Additionally, determining the process of sorting low-producing hens and assessing its impact on the welfare of the remaining flock are essential considerations. Research focused on incorporating technological tools in animal husbandry, providing standardised data through objective methods, could present groundbreaking opportunities in the future. Future research could explore temporal trends and integrate machine-learning techniques to refine predictive models. What seems likely to be realised in the near future is the identification of the best production methods for environmental and economic sustainability, and for increasing productivity while maintaining animal welfare and limiting harmful environmental impacts.

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## Authors' contributions

U.Ö.: Conception and design of the study, acquisition of data, and drafting of the manuscript. M.G.: Conception and design of the study, acquisition of data, and critical review/revision. E.L.: Analysis and/or interpretation of data and critical review/revision. Ö.Ç.: Conception and design of the study, analysis and/or interpretation of data, and critical review/revision. A.U.: Acquisition of data and critical review/revision. A.O.K.: Acquisition of data and critical review/revision.

## Conflict of interest declaration

The authors declare that they have no known competing financial interests or personal relationships that could appear to influence the work reported in this paper.

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