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Research article

Multi-response optimization of anti-GnRH immunocastration on growth performance and meat quality in weaned male guinea pigs

Jordan Ninahuanca[1,*], Josmel Castro-Carhuas[2], Edgar Garcia-Olarte[3], Carlos Quispe-Eulogio[1], Edith Ancco-Gomez[1] and Jose Luis Solís Rojas[4]

[1] Facultad de Ciencias de la Salud, Universidad Peruana Los Andes, Huancayo 12000, Peru
[2] Unidad de Producción Santa Ana, Sais Pachacútec Sac, Yauli 12780, Junín, Perú.
[3] Departamento Académico de Zootecnia, Universidad Nacional del Centro del Perú, Junín, Huancayo 12006, Perú.
[4] Departamento Académico de Ingenieria en Industrias Alimentarias, Universidad Nacional del Centro del Perú, Huancayo 12006, Av. Mariscal Castilla N° 3909, Junín, Peru

Abstract

This study evaluated the effects of different doses of an anti-GnRH immunocastrator on growth performance and meat quality in weaned male guinea pigs using a multi-response optimization approach. A total of 40 animals were randomly assigned to four treatments: one control group and three Bopriva® doses (0.20, 0.30, and 0.40 mL/animal), administered in two subcutaneous applications. Direct and derived productive traits, as well as physicochemical and color-related meat quality variables, were recorded. Statistical analysis included descriptive statistics, ANOVA, robust complementary tests, multivariate analysis of variance, principal component analysis, and the construction of a global decision index $I(\alpha) = \alpha P + (1-\alpha)Q$, where P represents the productive component and Q represents the meat quality component. The results showed that the intermediate dose achieved the best performance in growth and carcass weight, whereas the highest dose exhibited the best integrated meat-quality response and the highest global index value under a balanced scenario. The crossover point $\alpha^* = 0.522$ indicated that the optimal recommendation depended on the relative weight assigned to productivity and quality. It is concluded that T3 represents the best alternative when productive performance is prioritized, whereas T4 is more suitable when the main objective is to optimize meat quality and the overall balanced response.

Keywords: Decision threshold, Desirability function, Dose-response pattern, Phenotypic clustering, Postmortem acidification.

[*]**Corresponding author**: Jordan Ninahuanca, Facultad de Ciencias de la Salud, Universidad Peruana Los Andes, Huancayo 12000, Peru. Tel: 051-935937863. E-mail: d.jninahuanca@ms.upla.edu.pe

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INTRODUCTION

In Peru, guinea pig farming constitutes a livestock activity of profound economic, nutritional, and sociocultural relevance, especially in the Andean regions, where it forms part of traditional family production systems and, increasingly, of semi-intensive and commercial schemes oriented toward the market (Mulato-Sánchez et al., 2025; Donoso et al., 2025). Its importance lies not only in its contribution to the supply of high biological value protein for the population (Guerrero Pincay et al., 2020), but also in its role as a strategic resource for food security, income generation, and the sustenance of rural economies (Cuenca-Condoy et al., 2025). In this context, strengthening productive efficiency and guinea pig meat quality has become a technical and scientific priority (Sánchez-Macías et al., 2018), since both dimensions determine the competitiveness of the production system and the acceptance of the product in increasingly demanding markets. Therefore, the study of management strategies that make it possible to increase zootechnical performance without compromising animal welfare or product quality has gained special interest in applied research (da Costa Freitas and Barbosa, 2025).

Among these strategies, the castration of males has traditionally been used for management purposes, control of sexual behavior, reduction of aggressiveness, and, in some cases, improvement of certain carcass and meat attributes (Unchupaico et al., 2020). However, conventional surgical methods involve pain, stress, risk of infection, and growing concerns from the perspective of animal welfare (Marquette et al., 2023; Zalewska et al., 2026). In response to this, immunocastration emerges as a less invasive alternative, based on the induction of antibodies against gonadotropin-releasing hormone (GnRH) (Wang et al., 2023), with the aim of modulating the reproductive axis and its associated physiological effects (Ahmed et al., 2022; Lin et al., 2024). This approach has been widely explored in other livestock species of interest (Fan-Mei et al., 2022), but its evaluation in guinea pigs, especially under local production conditions, remains limited.

From a biological perspective, the response to an immunocastrator should not be interpreted solely in reproductive terms (Batorek et al., 2012). Endocrine modulation may have repercussions on growth, tissue deposition, metabolism, and, consequently, on productive variables such as final weight, weight gain, or carcass weight, as well as on meat quality attributes, including pH, proximate composition, and color (Pérez-Ciria et al., 2021; Poklukar et al., 2021). However, these responses do not necessarily evolve in the same direction. One dose may favor the productive component more strongly, whereas another may improve certain quality traits more markedly (Haddad et al., 2008). Consequently, the problem ceases to be strictly univariate and becomes a decision problem with multiple simultaneous responses (Augusto et al., 2012). This characteristic poses an important limitation in traditional approaches based exclusively on comparisons of means variable by variable. Although tests such as ANOVA make it possible to identify specific differences among treatments (Yu et al., 2022), they do not always allow a clear answer as to which dose is globally more convenient when the evaluation criteria are multiple and potentially conflicting. In other words, the optimal treatment does not necessarily coincide with the one that presents the highest value in a single variable (Asghari et al., 2022), but rather with the one that achieves the best compromise between productivity and quality. Under this logic, it is pertinent to complement the experimental evaluation with an integrative quantitative approach that transforms several responses into an explicit decision rule.

Recent studies have examined complementary aspects of livestock production, meat quality, and immunocastration in different animal species. Thirawong et al. (2024) evaluated growth performance, carcass traits, and meat quality in Wagyu crossbred cattle, reporting differences among genetic groups in

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productive performance, carcass characteristics, color traits, fat composition, and shear force. Lai et al. (2024) assessed the effect of Improvac® on sex hormones and reproductive organs in pigs, showing that anti-GnRH immunization reduced sex hormone concentrations and modified reproductive organ development. Similarly, Srisuwatanasagul et al. (2022) analyzed estrogen receptor alpha expression in the testes of immunocastrated fattening pigs according to the timing of GnRH vaccine administration. Although these studies provide valuable evidence on meat quality and immunocastration in livestock, none has evaluated anti-GnRH immunocastration in guinea pigs using a dose-response design, nor has integrated productive and meat quality traits within a multi-response decision framework. In this context, the present study contributes novel evidence by evaluating different doses of an anti-GnRH immunocastrator in weaned male guinea pigs and by interpreting the biological response through a multivariate and multi-response optimization approach. This strategy allows the identification of dose recommendations according to whether the production system prioritizes growth performance, meat quality, or a balanced compromise between both objectives.

Within this framework, the objective of the study was to evaluate the effect of different doses of an anti-GnRH immunocastrator on productive parameters and meat quality in weaned male guinea pigs, and to interpret the experimental response through a statistical and multivariate approach that allows identification of the most suitable dose according to a joint optimization criterion.

MATERIALS AND METHODS

All procedures performed on the guinea pigs were carried out in accordance with the provisions of Official Letter No. 002-GRJ-DRA-AAC-PERÚ-2025 and in compliance with the applicable national and international framework for animal care and welfare. In particular, the experimental management was conducted under principles aimed at minimizing pain, stress, and any form of unnecessary suffering.

Study Area

The study was conducted at the Yauris Agricultural Experimental Station of the Universidad Nacional del Centro del Perú, located in the district of El Tambo, province of Huancayo, department of Junín, Peru. This experimental unit is situated at an approximate altitude of 3204 m above sea level, in an environment representative of the central highlands of the country, a condition that gives it relevance for research aimed at high-Andean livestock production systems (Senamhi, 2025). The selection of this experimental station was based on its availability of infrastructure, controlled management conditions, and accessibility to animals under a productive system compatible with the objectives of the study.

Animals

A total of 40 clinically healthy weaned male guinea pigs of the Peru line (*Cavia porcellus*) were used. The animals were approximately (21–28 days old) at the beginning of the experimental management period and had an initial body weight ranging from 281 to 670 g, according to the individual baseline records. Before treatment allocation, all animals were individually identified, clinically examined, and managed under homogeneous housing, feeding, and sanitary conditions. The animals were randomly distributed into four experimental groups of 10 individuals each: a control group without immunocastrator (Table 1) and three groups treated with Bopriva® at total doses of 0.20 mL/animal (T2), 0.30 mL/animal (Table 3), and 0.40 mL/animal (Table 4), respectively. In the immunocastrated groups, the total dose corresponding to each treatment was divided into two subcutaneous applications of equal volume, administered in the dorsal cervical region (Unchupaico et al., 2020). Thus, the total doses of 0.20, 0.30, and 0.40 mL per

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animal corresponded to two successive applications of 0.10 + 0.10 mL, 0.15 + 0.15 mL, and 0.20 + 0.20 mL, respectively. Throughout the entire experimental period, the guinea pigs remained under homogeneous conditions of housing, feeding, sanitary management, and zootechnical supervision, with the aim of reducing external sources of variation and ensuring that the observed differences were attributed primarily to the effect of the treatment. The diet components were subjected to bromatological analysis at the Animal Nutrition Laboratory of the Universidad Nacional del Centro del Perú in order to determine their nutritional composition and characterize the dietary profile supplied to the experimental animals; these results are presented in Table 1.

Table 1 Feeding and nutritional contribution of the diet supplied to weaned guinea pigs.

Parameter	Fresh alfalfa (*Medicago sativa*)	Balanced feed
Feeding frequency	3 times/day	1 time/day
Daily supply proportion (%)	75.0	25.0
Dry matter (%)	21.00	-
TDN (%)	-	65
Crude protein (%)	20.14	18.00
Energy (Mcal/kg)	2.71 Mcal/kg	2.75 Mcal/kg
Crude fiber (%)	6.02	11.00
Calcium (%)	0.85	0.80
Phosphorus (%)	0.15	0.45
Drinking water	Continuous availability	-
TDN (%)	65	16.25
Crude protein (%)	18.00	4.50
Crude fiber (%)	11.00	2.75
Energy (Mcal/kg)	2.75	0.6875
Calcium (%)	0.80	0.20
Phosphorus (%)	0.45	0.1125

The dietary inclusion proportion indicates the relative contribution of fresh alfalfa and balanced feed in the daily ration on a fresh basis. Digestible energy is expressed as Mcal/kg of dry matter. Drinking water was available continuously throughout the experimental period.
*Nutritional contribution of the balanced feed when it represents the actual 25% of the ration.

Table 2 Verification of statistical assumptions and selection of the inferential method for productive variables and meat quality variables.

Variable	p	sig	Shapiro_p	Levene_p	p_welch	p_kw	parametric criterion
IW	0.089901	No	0.130273	0.531351	0.069029	0.088102	ANOVA
FBW	0.034149	Yes	0.340826	0.928534	0.061435	0.031587	ANOVA
WG	0.131877	No	0.37013	0.926541	0.163537	0.083514	ANOVA
ADG	<0.001	Yes	<0.001	0.133043	<0.001	<0.001	Kruskal/Welch
CW	0.017674	Yes	0.026092	0.105958	0.046917	0.021414	Kruskal/Welch
CY	0.017674	Yes	0.026092	0.105958	0.046917	0.021414	Kruskal/Welch
pH	<0.001	Yes	<0.001	0.003034	<0.001	<0.001	Kruskal/Welch
Moisture	<0.001	Yes	0.176746	0.055747	<0.001	<0.001	ANOVA
Fat	<0.001	Yes	0.165298	0.386007	<0.001	<0.001	ANOVA
Protein	<0.001	Yes	0.192988	0.984448	<0.001	<0.001	ANOVA
Ash	<0.001	Yes	0.193017	0.992339	<0.001	<0.001	ANOVA
L_star	<0.001	Yes	0.268185	0.934264	<0.001	<0.001	ANOVA
a_star	<0.001	Yes	0.01933	0.076087	<0.001	<0.001	Kruskal/Welch
b_star	<0.001	Yes	0.043858	0.051636	<0.001	<0.001	Kruskal/Welch

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Table 3 Explained variance and loadings of the first two principal components for productive and meat quality variables.

Block	Variable	PC1 loading	PC2 loading	PC1 variance (%)	PC2 variance (%)	Cumulative variance (%)
Productive block	Final body weight	0.707	-0.707	99.38	0.62	100.00
Productive block	Carcass weight	0.707	0.707	99.38	0.62	100.00
Meat quality block	pH	0.252	-0.499	55.31	28.69	84.00
Meat quality block	Moisture	-0.266	-0.422	55.31	28.69	84.00
Meat quality block	Fat	0.435	-0.210	55.31	28.69	84.00
Meat quality block	Protein	0.450	-0.186	55.31	28.69	84.00
Meat quality block	Ash	0.435	0.050	55.31	28.69	84.00
Meat quality block	L*	0.464	-0.040	55.31	28.69	84.00
Meat quality block	a*	0.179	0.482	55.31	28.69	84.00
Meat quality block	b*	0.195	0.508	55.31	28.69	84.00

PC1: first principal component; PC2: second principal component. Loadings indicate the contribution and direction of each variable in the construction of the principal components. Higher absolute values indicate greater contribution to the component. The sign of PCA loadings is arbitrary; therefore, the biological interpretation was based on the magnitude of the loadings and the relative direction among variables within each component. L*: lightness; a*: redness; b*: yellowness.

Table 4 Multi-response optimization indices and dose recommendation according to the decision criterion.

Treatment	P (mean ± SD)	Q (mean ± SD)	I(α=0.50) (mean ± SD)	Interpretation
T1	0.426 ± 0.211	0.397 ± 0.0730	0.411 ± 0.0762	Lowest overall performance
T2	0.461 ± 0.204	0.503 ± 0.00887	0.482 ± 0.101	Intermediate performance
T3	0.694 ± 0.270	0.439 ± 0.0365	0.566 ± 0.133	Best productive alternative
T4	0.572 ± 0.274	0.572 ± 0.0728	0.572 ± 0.105	Best quality alternative and best balanced option

represents the productive index, Qrepresents the meat quality index, and I(α=0.50) represents the global decision index under a balanced scenario between productivity and meat quality. SD: standard deviation. The crossover point between T3 and T4 was α^*=0.522.

Immunocastration protocol and Bopriva® treatment

The immunocastration protocol consisted of two subcutaneous applications administered in the dorsal cervical region. The first application was performed on April 1, 2025 (Day −14), and the second application was performed on April 15, 2025 (Day 0), which was considered the beginning of the productive evaluation period. Initial body weight was recorded on Day 0, immediately before the second application, and the productive evaluation continued for 56 days, until final body weight recording and slaughter on June 10, 2025. Therefore, the total experimental doses of 0.20, 0.30, and 0.40 mL/animal were divided into two equal applications of 0.10 + 0.10 mL, 0.15 + 0.15 mL, and 0.20 + 0.20 mL, respectively.

Bopriva® is an anti-GnRF immunocastration vaccine composed of a synthetic analogue of gonadotropin-releasing factor conjugated to an immunogenic carrier protein and formulated with an aqueous adjuvant. According to the product information used in this study, the vaccine contained 400 µg of GnRF-protein conjugate per mL. Accordingly, the total administered antigen equivalents were 80, 120, and 160 µg of GnRF-protein conjugate per animal for the 0.20, 0.30, and 0.40 mL/animal treatments, respectively. Since each total dose was divided into two applications, the antigen equivalents per application were 40 + 40 µg, 60 + 60 µg, and 80 + 80 µg per animal, respectively. The selected doses were defined as low, intermediate, and high exploratory levels adapted to the smaller body size of guinea pigs, because a standardized anti-GnRF immunocastration dose has not yet been established for this species. This dose-response design was intended to evaluate whether increasing levels of anti-GnRF immunization generated differential productive and meat quality responses.

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Production variable

The productive evaluation comprised three direct measurements: initial weight (IW), final body weight (FBW), and carcass weight (CW), all expressed in grams. Initial weight was recorded at the beginning of the productive evaluation period, coinciding with the second application of the immunocastrator, whereas final body weight was obtained at the end of the 56 experimental days. Carcass weight was determined after slaughter of the animals, once the corresponding carcass had been obtained. From these measurements, three derived variables were calculated: weight gain (WG), estimated as the difference between final body weight and initial weight (WG = FBW - IW) (Carhuas et al., 2024); average daily gain (ADG), calculated by dividing weight gain by the duration of the experimental period (ADG = (FBW - IW)/56); and carcass yield (CY), determined as the percentage ratio between carcass weight and final body weight (CY = (CW/FBW) × 100) (Carhuas et al., 2023). Taken together, these variables made it possible to characterize the productive response of the animals both in terms of body growth and final carcass performance.

Meat quality

Meat quality was evaluated after slaughter using physicochemical indicators, proximate composition, and instrumental color parameters. Postmortem pH was measured 24 h after slaughter, after carcass chilling at approximately 4 °C, in the Biceps femoris muscle from the hind limb using a Starter 3100 digital pH meter. Before measurement, the pH meter was calibrated with standard buffer solutions at pH 4.00 and 7.00. For each carcass, three readings were recorded at different points of the same muscle, and the mean value was used for statistical analysis.

Proximate composition included moisture, fat, protein, and ash, all expressed as percentages and determined through bromatological analysis from samples of the same muscle collected after the 24 h chilling period. Protein was quantified by the Kjeldahl method, ash by muffle furnace incineration, and moisture and fat by standardized laboratory procedures (Ts'ephe et al., 2024).

Instrumental color was measured 24 h postmortem on the freshly exposed surface of the Biceps femoris muscle using an LS175 colorimeter. Before color measurement, the muscle surface was exposed to air for approximately 30 min to allow blooming. The CIE Lab parameters L, a, and b* were recorded, corresponding to lightness, redness, and yellowness, respectively (Dzurenda, 2023). For each sample, three readings were taken at different points of the muscle surface and averaged before statistical analysis.

Statistical Analysis

The database was previously cleaned and verified. In the descriptive phase, the variables were summarized by mean, standard deviation, median, range, and coefficient of variation by treatment. Outliers were identified using the interquartile range criterion, although all observations were retained after verifying their biological plausibility and the absence of recording errors. For each response variable, a one-factor linear model corresponding to the treatment effect was initially fitted:

$$Y_{ij} = \mu + \tau_i + \varepsilon_{ij}$$

where Yij represents the observed value of the response variable in animal j under treatment i, μ is the overall mean, τi is the fixed effect of treatment i, and εij is the random error term, assumed to be independently distributed with mean zero and constant variance. Prior to parametric inference, the assumptions of residual normality were assessed using the Shapiro–Wilk test and homogeneity of variances using Levene's test. When the assumptions were reasonably satisfied, differences among treatments were evaluated by one-way analysis of variance (ANOVA), and multiple comparisons were performed using Tukey's test. For those variables in

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which the parametric assumptions were compromised, the robustness of the inference was assessed using Welch's ANOVA and the nonparametric Kruskal–Wallis test. Since initial weight could influence final body weight and carcass weight, both variables were additionally analyzed by analysis of covariance (ANCOVA), using initial weight as a covariate. The fitted model was:

$$Y_{ij} = \mu + \tau_i + \beta(X_{ij} - \bar{X}) + \varepsilon_{ij}$$

where Xij represents the initial body weight of animal j under treatment i, $\bar{X}$ is the overall mean initial body weight, and β is the regression coefficient associated with the covariate. Before applying this adjustment, the homogeneity of slopes was verified through the treatment × initial weight interaction. As no significant interaction was detected, the classical ANCOVA was carried out, and adjusted means by treatment were estimated together with their multiple comparisons.

In order to evaluate the joint response of several variables, a multivariate approach was also implemented. In the productive block, FBW and CW were included, whereas in the meat quality block, pH, moisture, ash, fat, protein, and the chromatic parameters L*, a*, and b* were considered. The overall significance of treatment was assessed primarily by Wilks' lambda (Λ) and Pillai's trace. Additionally, the association structure among variables and the multivariate separation among treatments were explored through principal component analysis (PCA), based on standardized variables as follows:

$$Z_{ij} = \frac{X_{ij} - \bar{X}_j}{s_j}$$

where Z_{ij} is the standardized value of variable $j$in animal i, X_{ij} is the original observed value, $\bar{X}_j$ is the mean of variable j, and s_j is its standard deviation. This transformation made it possible to eliminate the effect of the different measurement scales and to facilitate the interpretation of the loadings of the principal components.

Finally, in order to integrate the productive and meat quality responses into a single decision rule, a multi-response optimization scheme based on linear desirability transformations was implemented. Each response variable was transformed to a common dimensionless scale ranging from 0 to 1, where values closer to 1 indicated a more desirable response. For variables in which higher values were considered more favorable, the following maximization function was used:

$$d_{ij}^{(+)} = \frac{x_{ij} - m_j}{M_j - m_j}$$

where $d_{ij}^{(+)}$ represents the desirability value for animal i in variable j, x_{ij} is the observed value, m_j is the minimum observed value of variable j, and M_j is the maximum observed value of variable j.

For variables in which lower values were considered more favorable, the following minimization function was used:

$$d_{ij}^{(-)} = \frac{M_j - x_{ij}}{M_j - m_j}$$

where $d_{ij}^{(-)}$assigns higher desirability values to lower observed values of variable j. In both transformations, the least desirable observed value received a score of 0, whereas the most desirable observed value received a score of 1.

Subsequently, a productive index P_i and a quality index Q_i were constructed as weighted combinations of the individual desirabilities:

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$$P_i = \sum_{j=1}^{p} w_j^{(P)} \; d_{ij}^{(P)}$$

$$Q_i = \sum_{k=1}^{q} v_k^{(Q)} \; d_{ik}^{(Q)}$$

Where $w_k^{(Q)}$ are the weights assigned to each variable, and $d_{ij}^{(P)}$ and $d_{ik}^{(Q)}$ are the corresponding desirability values. Both indices were integrated into a global decision function defined as:

$$I_i(\alpha) = \alpha P_i + (1-\alpha) Q_i; \quad 0 \leq \alpha \leq 1$$

Where α represents the relative priority assigned to the productive component and $1-\alpha$ the priority of the quality component. The sensitivity of this function was evaluated by varying α between 0 and 1, in order to identify the optimal treatment under different decision scenarios. Additionally, the stability of the global indices was examined through bootstrap resampling and analysis of alternative weighting scenarios. In all analyses, a significance level of $p < 0.05$ was adopted, and the statistical processing was performed in the R environment (R Core Team, 2023).

RESULTS

Descriptive characteristics

From a descriptive perspective, treatment T3 presented the highest mean values in most of the main productive indicators, including initial weight (579.3 ± 67.61 g), final body weight (1190.8 ± 160.59 g), weight gain (611.5±117.14 g), average daily gain (10.92 ± 2.09 g/day), and carcass weight (791.4 ± 107.15 g), suggesting a favorable trend in the growth component in this group. In contrast, carcass yield was descriptively higher in T2 (70.52 ± 0.02%), a variable that also showed very low variability across all treatments. In the meat quality block, pH was highest in T1 (6.167 ± 0.073) and lowest in T4 (6.025 ± 0.077); moisture reached its highest mean in T2 (11.409 ± 2.719%); fat was highest in T1 (10.831 ± 1.323%) and lowest in T2 (6.857 ± 1.356%); whereas protein, ash, and lightness (L*) also showed their highest means in T1. In turn, the chromatic parameters showed differentiated profiles among treatments, with T3 standing out in a* (5.199 ± 0.677) and T4 in b* (6.510 ± 0.963). In terms of dispersion, the coefficients of variation were generally moderate for the productive variables, whereas some quality variables, especially in T2, showed greater relative heterogeneity (Figure 1).

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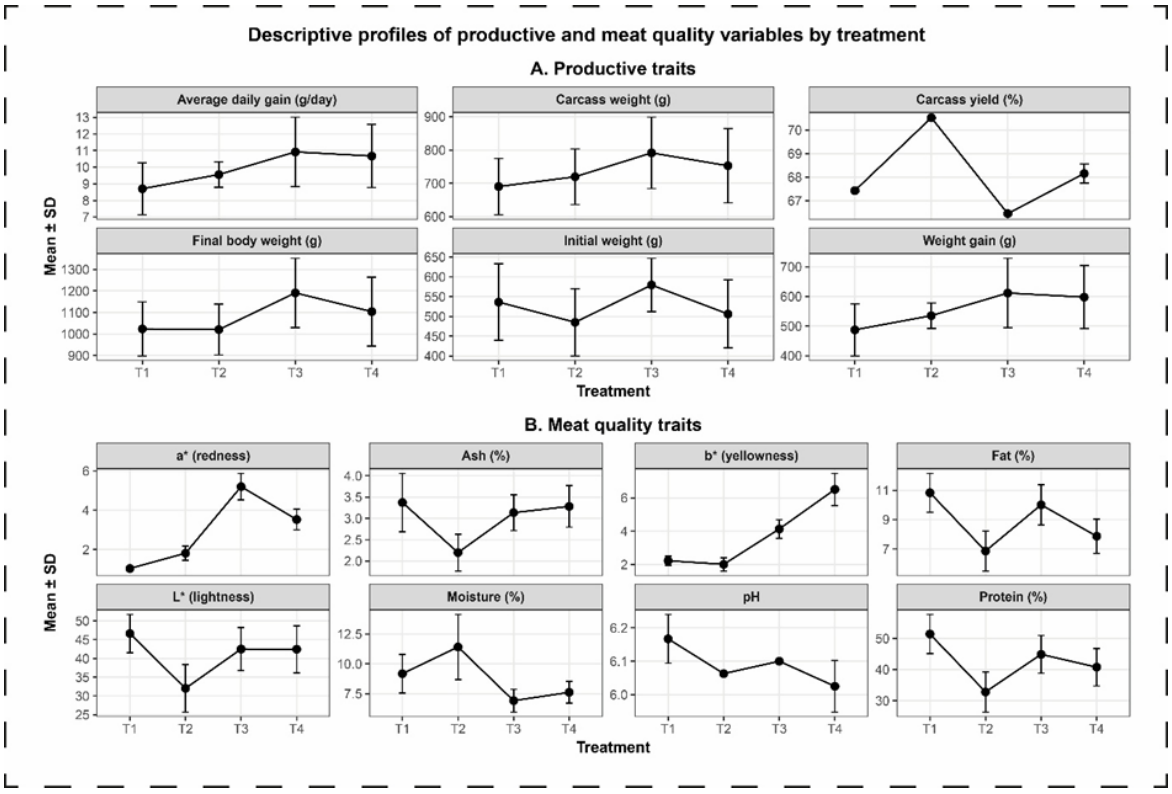


Figure 1 Mean and standard deviation by treatment for production variables, production derivatives, and meat quality.

Principal component analysis (PCA)

The PCA interpretation was revised by incorporating the loadings of the first two principal components, allowing the contribution of each variable to be identified and the biological meaning of each axis to be clarified (Table 3; Figure 2). In the productive block, PC1 explained 99.38% of the total variance, whereas PC2 explained only 0.62%. The loadings of final body weight and carcass weight on PC1 were identical and positive (0.707 and 0.707, respectively), indicating that this component represented a common axis of body growth and carcass size. Therefore, the productive PCA should not be interpreted as evidence of a complex multidimensional structure, but rather as a graphical and quantitative confirmation of the strong collinearity between final body weight and carcass weight. In this context, the added value of the productive PCA was to summarize both productive traits into a single dominant dimension of productive performance.

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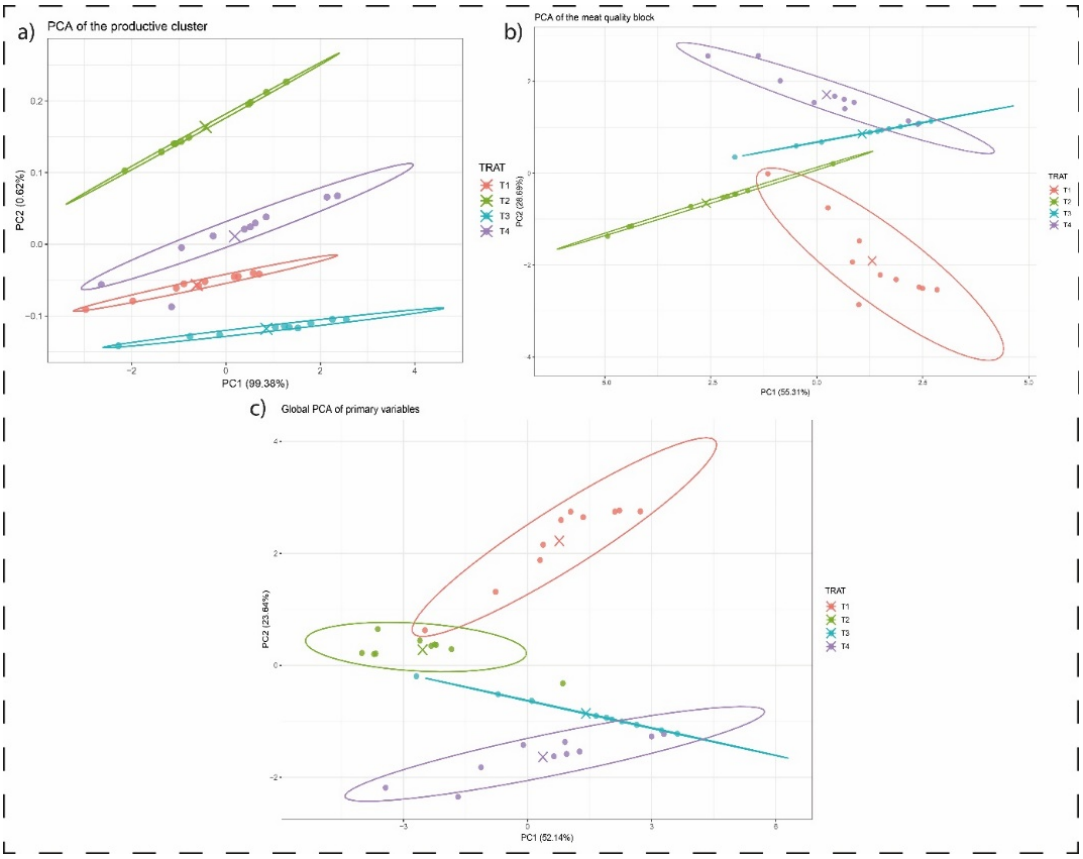


Figure 2 Principal component analysis (PCA) of the evaluated variable blocks in weaned guinea pigs: (a) productive block, composed of final body weight (FBW) and carcass weight (CW); (b) meat quality block, composed of pH, moisture, ash, fat, protein, lightness (L*), redness (a*), and yellowness (b*); and (c) global PCA constructed from final body weight (FBW), carcass weight (CW), pH, moisture, ash, fat, protein, lightness (L*), redness (a*), and yellowness (b*).

In the meat quality block, PC1 and PC2 explained 55.31% and 28.69% of the total variance, respectively, accumulating 84.00% of the total variability. This result indicates that meat quality was not represented by a single dominant component, but by at least two biologically relevant dimensions. PC1 was mainly defined by positive loadings for L* (0.464), protein (0.450), fat (0.435), and ash (0.435), together with a negative loading for moisture (-0.266). Therefore, PC1 can be interpreted as a compositional and lightness-related axis, in which higher scores reflect meat samples with greater proximate component concentration and higher lightness, whereas lower scores are more associated with moisture. PC2 was mainly characterized by positive loadings for b* (0.508) and a* (0.482), and negative loadings for pH (-0.499) and moisture (-0.422). Thus, PC2 can be interpreted as a chromatic intensity axis contrasting redness and yellowness with pH and moisture-related variation.

The global PCA, which jointly included productive and meat quality variables, was considered complementary because it provided an exploratory visualization of the overall response pattern among treatments. However, the block-specific PCA offered a clearer biological interpretation: the productive response was essentially unidimensional, whereas meat quality showed a more complex structure involving proximate composition, lightness, chromatic traits, pH, and moisture. Overall, these findings indicate that PCA was useful not only for visualizing treatment

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separation, but also for identifying the underlying structure of the productive and meat quality responses.

Multi-response optimization

The multi-response optimization indices constructed from the productive and quality components showed a differentiated pattern among treatments (Table 4). Treatment T3 presented the highest mean value of the productive index (P = 0.694 ± 0.270), whereas T4 reached the highest value in the quality index (Q = 0.572 ± 0.0728) and also in the balanced global index. These results indicate that T3 preferentially maximizes the productive component, whereas T4 offers the best integrated response under a balanced criterion between productivity and quality. The crossover value α^*=0.522 confirmed that the optimal recommendation depends on the relative weight assigned to each objective: when productivity exceeds 52.2% of relative importance, T3 becomes the preferable alternative; in contrast, under a greater priority of the quality component, T4 emerges as the best option. The behavior of the multi-response optimization index varied according to the decision criterion (Figure 3). In the balanced scenario (α_m=0.50), the global index showed its highest value in T4, followed very closely by T3, whereas T1 presented the lowest integrated performance. When the sensitivity of the index was evaluated under different external weightings, a consistent pattern was observed: T4 maintained the best response in balanced and quality-oriented scenarios, whereas T3 reached the highest mean index value in the scenarios with greater productive priority. T2 and T1 remained systematically below both treatments in all the schemes evaluated. Finally, the heatmap of the winning treatment confirmed that the optimal decision was concentrated exclusively between T3 and T4, with neither T1 nor T2 emerging as superior alternatives in any scenario. Taken together, these results demonstrate that the optimal dose was not absolute, but rather dependent on the relative weight assigned to the productive and quality components, reinforcing the relevance of the multi-response optimization approach adopted in this study.

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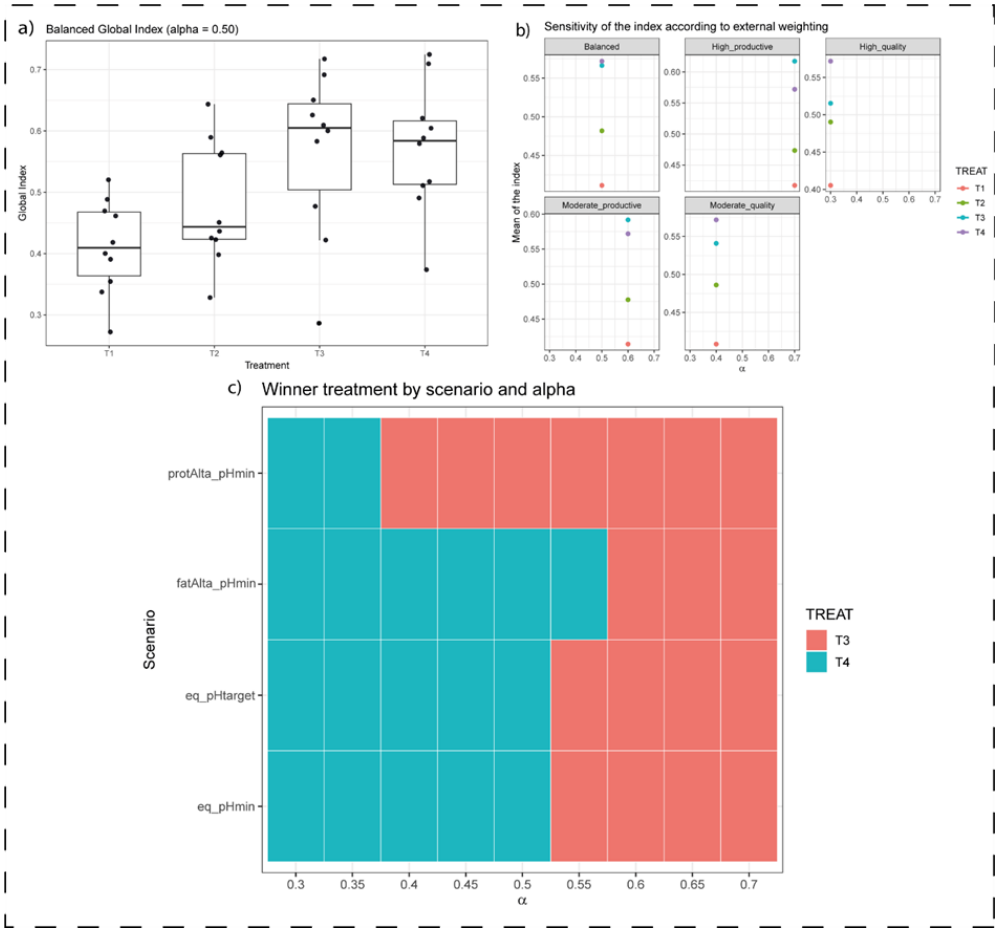


Figure 3 Multi-response optimization of the experimental treatments. a) Balanced global index. b) Sensitivity according to external weighting. c) Winning treatment under different decision scenarios.

DISCUSSION

The results of the present study show that anti-GnRH immunocastration in weaned male guinea pigs was associated with a differentiated response between the productive component and meat quality, indicating that the interpretation of treatment effects should not be reduced to a single variable or to a single "best treatment." In the productive block, T3 presented the highest mean values for final body weight, weight gain, average daily gain, and carcass weight, suggesting that the intermediate dose generated the most favorable productive profile under the conditions of this experiment. This pattern is consistent with the general literature on immunocastration, in which the response to GnRH blockade has been reported to depend on species, age, immunization frequency, physiological status, and the intensity of the endocrine response (Batorek et al., 2012). However, because reproductive hormones or anti-GnRH antibody titers were not measured in the present study, the endocrine interpretation should be considered a plausible explanatory hypothesis rather than a confirmed mechanism. Likewise, previous studies indicate that immunocastration may influence growth performance and carcass traits, although these effects are not necessarily linear or uniform across all levels of intervention (Poklukar et al., 2021; Pérez-Ciria et al., 2021). However, the productive advantage observed in T3 was not fully reflected in the meat quality block. In this component, T4 showed a more competitive profile, particularly due to its lower mean pH, higher b* value, and higher values in both the quality index Q

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and the balanced global index I(α=0.50). This result suggests that the highest dose did not necessarily maximize growth performance but may have been more closely associated with changes in technological and visual meat quality attributes (Ahmed et al., 2022). In meat science, postmortem pH and the CIELAB coordinates constitute relevant quality indicators, since pH is associated with color stability, water-holding capacity, and the final appearance of the product, whereas L*, a*, and b* summarize visual characteristics directly related to commercial acceptance (Sristi et al., 2025). Therefore, the response observed in T4 should be interpreted as evidence of a differentiated quality profile rather than as proof of a specific physiological mechanism.

The applied interpretation of the crossover point α*=0.522 helps to summarize the decision boundary between productive performance and meat quality. When the priority assigned to productivity exceeds 52.2%, T3 becomes the most suitable alternative; in contrast, when quality receives greater relative weight, T4 becomes the preferable option. This finding has practical relevance because it indicates that the optimal dose is not an intrinsic or universal property of the treatment, but rather depends on the decision criterion adopted (Ahmed et al., 2022). From a production-oriented perspective, systems seeking to maximize growth and carcass weight would tend to favor T3, whereas systems oriented toward a more balanced response or with greater emphasis on meat quality could justify the selection of T4. In this sense, the study not only identifies differences among doses, but also translates those differences into an explicit decision rule. The observed pattern may be compatible with a nonlinear dose-dependent response to anti-GnRH immunocastration (Asghari et al., 2022; Yu et al., 2022). Nevertheless, the biological basis of this response should be interpreted with caution, because the present study did not include direct measurements of reproductive hormones, anti-GnRH antibody titers, metabolic indicators, or postmortem muscle biochemical dynamics. Thus, although the higher productive response observed in T3 may be compatible with a more favorable balance between immunological stimulation and growth performance (Unchupaico et al., 2020), this should be interpreted only as a hypothesis, because the study did not quantify endocrine suppression, antibody response, or metabolic redistribution. Similarly, the advantage of T4 in the quality block may suggest an association between the highest dose and meat physicochemical or chromatic traits, but it cannot be interpreted as evidence of stronger gonadal-axis suppression or altered postmortem muscle metabolism without direct hormonal, immunological, or biochemical measurements (Pérez-Ciria et al., 2021). Since final pH is a key determinant of meat color and visual quality, the differences observed in pH and CIELAB parameters are biologically plausible; however, future studies should incorporate endocrine, immunological, and muscle metabolism markers to confirm the mechanisms underlying these treatment responses.

These findings are also consistent with the general literature that describes immunocastration as a less invasive alternative to surgical castration (Ahmed et al., 2022; Wang et al., 2023; Lin et al., 2024). Recent reviews indicate that anti-GnRH immunocastration can reduce some welfare concerns associated with surgical procedures and may influence performance, carcass traits, and meat quality; however, the magnitude and direction of these effects depend strongly on the species, vaccination protocol, age, production system, and evaluated traits (Ubilla et al., 2025; Luo et al., 2026). Within this framework, the results obtained in guinea pigs contribute preliminary evidence that different doses may favor different response dimensions, with T3 more closely associated with productive performance and T4 with the integrated quality response. Therefore, any endocrine-based explanation proposed in this study should be understood as a biologically plausible hypothesis derived from the known mode of action of anti-GnRH immunocastration, and not as a mechanism directly demonstrated by the present experimental data. However, these findings should be regarded as an exploratory dose-response evaluation rather than as a definitive physiological

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characterization of immunocastration in this species. Therefore, the responses observed in T3 and T4 are better understood as complementary treatment profiles within the same experimental framework, rather than as contradictory results. This reinforces the usefulness of the multi-response optimization approach, since it allows dose recommendations to be aligned with the specific objective of the production system, whether focused on growth, meat quality, or a balanced compromise between both components.

CONCLUSIONS

In the productive component, the intermediate dose of the anti-GnRH immunocastrator (T3) showed the most favorable response, recording the highest values of final body weight, weight gain, average daily gain, and carcass weight, indicating that this treatment level optimized growth and final performance in weaned male guinea pigs. In the meat quality component, the highest dose (T4) presented the most competitive profile, achieving the highest quality index and the best overall performance under a balanced criterion, evidencing advantages in physicochemical and chromatic attributes of technological and commercial relevance. From an analytical standpoint, the use of ANOVA, MANOVA, PCA, and the multi-response optimization index $I(\alpha) = \alpha P + (1 - \alpha)Q$ made it possible to demonstrate that the optimal dose is not absolute, but rather dependent on the decision criterion; in particular, the crossover value $\alpha^*=0.522$ indicated that T3 is preferable when productive priority exceeds 52.2%, whereas T4 is more suitable when greater relative weight is assigned to meat quality.

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CONFLICT OF INTEREST

There is no conflict of interest with the publication of this article. The authors declare that Artificial Intelligence (AI) tools, specifically ChatGPT by OpenAI, were used only as an auxiliary tool to support grammar refinement, clarity, readability, and fluency in certain specific points of the manuscript, not throughout the entire manuscript. The AI tool was not used to write or draft the manuscript text, generate scientific content, fabricate, falsify, manipulate, or alter data, statistical results, figures, tables, images, references, or scientific conclusions. All sections of the manuscript were critically reviewed, validated, and approved by the authors, who take full responsibility for the integrity, accuracy, originality, reproducibility, and scientific content of the manuscript.

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