

Application of PLS-SEM in assessment of the effect of animal welfare

MEHMET SARAÇOĞLU, SABRİ YURTSEVEN, HAMZA YALÇIN

Department of Animal Science, Faculty of Agriculture, Harran University, Şanlıurfa, Türkiye

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Saraçoğlu M., Yurtseven S., Yalçin H.

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Summary

The objective of this study was to determine the impact of welfare on growth, physical activity, biological stress, physiological stress, biological variables, and the immune system in Japanese quails. Moreover, the study aimed to evaluate the practical applicability of these latent variables within the framework of welfare modeling in Japanese quails. To achieve these objectives, a data set comprising blood variables from 126 Japanese quails was analyzed using partial least squares structural equation modeling (PLS-SEM). The model employed a hierarchical component model (HCM), where welfare was designated as the high-order component (HOC), while physiological variables were treated as lower-order components (LOC). The results of the modeling revealed that welfare significantly influenced physical activity, immune system, and growth in Japanese quails, with path coefficients of 0.791, 0.567, and 0.903, respectively. The model also yielded R^2 values for the endogenous latent variables, indicating that welfare explained 62.6% of the variance in physical activity, 32.1% in the immune system, and 81.6% in growth. These findings underscore the importance of welfare in shaping key physiological and biological outcomes in Japanese quails. The study not only enhances our understanding of how welfare impacts these variables but also provides a robust framework for predicting the effects of animal welfare on related latent variables in Japanese quail.

Keywords: welfare, modeling, quail, physiological variables, PLS-SEM

The demand for meat has surged in tandem with the rapid growth of the world population, with a particular demand is for poultry meat due to its low-calorie content, high protein levels, and low cholesterol (6, 32, 36). This increased demand has, however, placed significant pressure on the agricultural sector to enhance efficiency, often at the expense of environmental and ethical standards. As a result, the welfare of poultry has come under scrutiny (7, 27).

Improving poultry welfare is not only essential for the health and well-being of the birds themselves but also directly impacts the quality of the meat produced. Ensuring optimal welfare conditions is, therefore, critical for producing a high-quality product (14, 33). Consequently, there is growing recognition that evaluating and enhancing animal welfare is a priority. This task demands the collaboration of scientists, policymakers, and industry stakeholders, all of whom play crucial roles in advancing standards that benefit both animals and broader agricultural system (4, 13, 41).

Animal welfare must be objectively assessed and scientifically validated to improve conditions for animals and ensure the industry's long-term sustainability

(60). The concept of animal welfare encompasses an animal's biological responses to its external environment. It can be measured through various indications, including physiology, emotional and cognitive state, avoidance behavior, biochemistry, and reactions to physicochemical or social stimuli (28, 62). However, while these extrinsic or objective metrics can offer valuable insight, they can not directly reveal the mental states that constitute an animal's subjective well-being. Instead, physiological, or behavioral changes serve as proxies, providing evidence of an animal's welfare (5). Physiological stress, in particular, is a natural biological response when an animal's welfare is compromised (20, 35, 53, 59).

There are various methods for assessing animal welfare, each with its own advantages (43). However, recent advancements in statistical and mathematical modeling, as well as the application of machine learning and data mining, offer promising new avenues for understanding these complex processes. These techniques are increasingly being integrated into perspective analytics algorithms for animal health and welfare (46). To this end, various algorithms have

been developed to identify measurable indicators that explain the greatest variance in latent variables. This approach allows for the reduction of the number of indicators that need to be recorded simultaneously, thereby streamlining the assessment process (25). One of the most powerful methods in this regard is structural equation modeling (SEM). SEM enables researchers to model and predict complex relationships between multiple dependent and independent variables simultaneously. The concepts being examined in SEM are often unobservable and indirectly measured by multiple indicators. By accounting for measurement error in the observed variables, SEM provides a more accurate estimation of the underlying factors that influence animal welfare.

PLS-SEM (Partial Least Squares Structural Equation Modeling) has gained popularity as a method for estimating path models with latent variables and their relationships (50). It is rapidly emerging powerful statistical modeling technique (26). PLS-SEM has been widely applied across various scientific disciplines and is increasingly finding its place in new fields. For example, it has been used in retailing and consumer services (17), environmental science (11), sustainable entrepreneurship (52), education and information technology (67), economics (16) and medicine (66). This versatility underscores its growing relevance in both established and emerging research areas.

The earliest models were developed with the economic sector in mind (19). However, a substantial body of work in agricultural science has also focused on measuring latent variables. For instance, models have been employed to predict farm productivity (39) and to explore how positive emotions affect the physiological and behavioral characteristics of growing pigs (34). Despite these advances, there remains a notable gap the application of such models in the realm of animal welfare. Research on creating indicators for the welfare of sows and piglets (19) and the evaluating the welfare of poultry (65) is still relatively scarce. This suggests that the full potential of statistical models has yet to be realized in the field of animal welfare.

In this study, we investigated the effects of welfare on certain blood variables in Japanese quail (*Coturnix coturnix japonica*) that have not been previously explored in the literature. To conduct this analysis, we employed PLS-SEM. The study focused on animal welfare and the related latent variables: physical activity (PA), immune system (IS), and growth (GR).

Physiological indicators can provide crucial insights into the health and welfare of animals. By using this model, it is possible to explore how the exogenous latent variable, PA, affects levels of total cholesterol (TC), low-density lipoprotein (LDL), and high-density lipoprotein (HDL), offering valuable and detailed information (12). In avian species, the immune system's functionality relies on key antibody classes, including immunoglobulin-M (IgM), immunoglobulin-A (IgA),

and immunoglobulin-Y (IgY), which are similar to immunoglobulin-G (IgG) in humans (63). Since serum levels of IgG, IgM, and IgA are produced humoral immune cells within the body, they serve as a reliable indicator of the immune system's status. Therefore, assessing these variables can provide essential information on an individual's immune health (57).

The GR variable in this study included two key components: total phosphorus (TP) and calcium (Ca). Calcium is particularly vital for the proper growth of chickens. Beyond being a fundamental component of bone tissue and eggshell formation, Ca plays an essential role in regulating acid-base balance and supporting enzymatic systems (64). Protein, another critical factor, serves as the primary transporter of physiologically active substances. It is essential not only for body growth and tissue regeneration but also for the synthesis of proteins that build vital substances like hormones and enzymes, which are indispensable to the physiology of all living organisms (3). Therefore, adequate protein levels are crucial for overall growth and the maintenance of essential bodily functions.

Material and methods

Ethics. The ethical protocol for this study was reviewed and approved by the Harran University Animal Experiments Local Ethics Committee (Şanlıurfa, Türkiye), on 03/09/2022 with approval number 23022.

Housing Quails and Experimental Design. A total of 315 one-day-old Japanese quail (*Coturnix coturnix japonica*) were used in this study. The quails were selected to have equal average weights and were housed in cages measuring 50 × 50 × 50 cm, with 15 quails per cage. The quails were kept under controlled conditions at a temperature of 24°C ± 2°C and a 23 : 1 h light-dark cycle. They were provided with unrestricted access to water and feed. To acclimatize them to the experimental diet, the quails were fed a starter feed for one week. The feeding trial lasted for 6 weeks (42 days). The composition and nutrient content of the basal diets are detailed in Table 1.

Blood Constituents. Following the completion of the experimental procedure, a total of 126 quails, 6 quails (3 males and 3 females) from each cage were selected for the evaluation of blood variables. The appropriate techniques for measuring these variables were employed in accordance with relevant standards. Approximately 5 ml of blood was collected from each selected bird by severing the jugular veins. The blood samples were then centrifuged at 3,000 rpm for 15 minutes to separate the serum. The blood variables, including total cholesterol (TC), low-density lipoprotein (LDL), high-density lipoprotein (HDL), calcium (Ca), and total protein (TP), were measured spectrophotometrically using a Siemens Healthineers Atellica analyzer (Erlangen, Germany). The value of very low-density lipoprotein (VLDL) was calculated using the Friedewald formula, which determines TC by summing LDL, HDL and VLDL values (18). Immunoglobulin levels (IgA and IgM) were measured using the nephelometric method with a Siemens BN2 analyzer (Erlangen, Germany). Hematological

Tab. 1. The feed composition and nutritional content of the basal diets

Calculated Composition	Basal Diet	
	¹ Starter (%)	² Grower (%)
Yellow Corn	50.00	54.00
Soybean meal (44% CP)	18.18	7.60
Maize gluten meal (43% CP)	12.00	11.50
Sunflower meal (45% CP)	6.50	8.00
Soybean, Full Fat	8.50	7.90
Di-calcium phosphate	1.05	0.70
CaCO ₃	1.02	2.00
Salt NaCl	0.40	0.30
Premix	0.30	0.34
Limestone	1.30	4.00
DL-Methionin	0.20	0.30
L-Lysine	0.55	0.36
Total	100.00	100.00
Calculated analysis		
ME (kcal/kg)	2906	2900
Crude Protein (%)	24.0	20.1
Calcium (%)	1.26	2.55
Methionine + cysteine (%)	1.04	1.05
Lysine (%)	1.46	1.05
Available phosphorus (%)	0.30	0.22

Explanations: ¹Premix provides per kg of starter diet: Vit A – 18.0 IU; Vit D3 – 3.96 IU; Vit E – 30 IU; Cobalt – 0.15 mg; Copper – 15.90 mg; Iodine – 0.60 mg, Iron – 205.86 mg; Manganese – 140.11 mg; Selenium – 0.38 mg, Zinc – 125.27 mg. ²Premix provides per kg of grower diet: Vit A – 20.40 IU; Vit D3 – 4.49 IU; Vit E – 30.0 IU; Cobalt – 0.17 mg; Copper – 15.37 mg; Iodine – 0.68 mg, Iron – 299.95 mg; Manganese – 156.84 mg; Selenium – 0.40 mg, Zinc – 134.32 mg.

values, including platelet count (PLT), mean platelet volume (MPV), and procalcitonin (PCT) were determined using an Abbott Diagnostics hematology analyzer (Santa Clara, California, USA). Commercial kits were used for all these measurements.

Statistical Analysis

Partial least squares structural equation modeling (PLS-SEM). Partial least squares (PLS-SEM) and Covariance-Based (CB-SEM) Structural Equation Modeling are commonly employed methodologies for evaluating complex theoretical relationships between multiple variables. It is particularly effective with smaller sample sizes, does not require normally distributed data, and easily integrates into formalized (composite) measurable constructs (21). PLS-SEM combines elements of factor analysis with regression, making it especially suitable for research focused on causal relationships and transition paths. The approach uses an iterative process to quantify and model the structure of dependent variables, minimizing discrepancies between the dependent and independent variables. Additionally, the PLS-SEM allows for the examination of crucial inter-relationships among a set of variables, providing deeper insights into underlying dynamics (22, 29).

In the SEM, variables are categorized into two types: latent variables, which are not directly measurable, and latent measurement variables, which are indicators that predict or reflect the latent variables. A SEM consists of two main components: measurement models, which include latent measurement variables and their associated indicators, and a structural model, which involves endogenous and exogenous latent variables. Endogenous latent variables are influenced by other latent variables, whether they are exogenous or endogenous. In contrast, endogenous latent variables are independent and not affected by other latent variables. The coefficient of determination (R^2) is calculated solely for endogenous latent variables, indicating the extent to which a latent variable is explained by other latent variables. Moreover, endogenous latent variables can influence other endogenous constructs and can also serve as exogenous latent variables in certain contexts. In the structural model, path coefficients – represented by arrows – depict the relationship between endogenous and exogenous latent variables. These coefficients range from -1 to $+1$, with the arrows pointing from exogenous latent variables to endogenous latent variables, illustrating the direction and strength of the relationships.

The SEM employed in this study consists of four latent variables: animal well-being, physical activity, immune system, and growth. Data were collected exclusively from the animals at the times of slaughter, following the fattening period (Tab. 2). The structural model was utilized to perform regression and significance analyses to ascertain the pathways through which the welfare component influences the variance of the other three groups of variables. This analysis aims to clarify how animal welfare impacts these factors.

Our primary focus was on the R^2 values, which indicates the extent to which the well-being component accounts for the variability in the other three groups of latent variables. Additionally, the coefficient f^2 was employed to measure the effect size of the exogenous latent variables on the endogenous latent variables. The evaluation of f^2 is based on the specific thresholds: a value of 0.02 indicates a modest effect, 0.15 indicates a medium effect, and 0.35 signifies a large effect of the exogenous latent variable (10, 24).

Hierarchical Component Models (HCM). To gain a deeper understanding of the complex structures analyzed in this study, a hierarchical component model (HCM) with two levels of abstraction was constructed. HCMs are crucial for assessing complex systems that go beyond simple first-order structures, involving more than one level of organization. This specific HCM is composed of two levels: the higher-level component (HOC) and the lower-level components (LOC). The HOC evaluates the broader conceptual level, while the LOC represents the sub-dimensions of the main component.

In this study, the welfare status of Japanese quail was identified as the HOC, with other characteristics categorized as the LOC. A reflecting-reflective HCM was employed, where a reflective interaction occurs between the higher-order and lower-order clusters. This Means that all elements of the LOC are based on measurement models, in which the indicators represent the underlying construct, and the

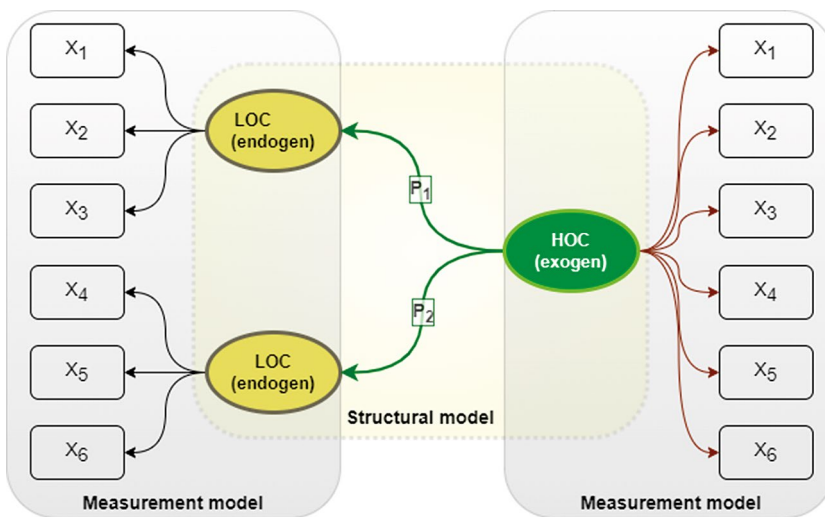


Fig. 1. Reflective-reflective hierarchical component model (HCM). In SEM diagrams, circles depict latent variables, rectangles represent indicators, arrows between circles show relationships (path coefficients), and arrows from circles to rectangles indicate indicator reliabilities

causality is directed from the construct to the indicators. Significantly, when developing the HOC measurement model, all indicators of the LOC were also associated with the HOC, following the framework established by Hair et al. (21) (Fig. 1). This hierarchical approach allowed for a comprehensive examination of the intricate structures under investigation, providing deeper insights into the relationships between welfare status and measured characteristics.

Evaluation of SEM

Evaluation of the measurement model. The primary objective of evaluating a PLS-SEM is to ensure its reliability and validity. Internal consistency reliability is typically assessed using composite reliability (CR) and Cronbach's alpha. In general, Cronbach's alpha and CR values are considered acceptable if they are equal to or greater than 0.7. To assess the convergence validity efficiency of a construct, the average variance extracted (AVE) is used. The AVE is calculated by averaging the variances of all terms within each construct. According to Hair et al. (21), an AVE value should typically exceed 0.5 to indicate satisfactory convergence. Another important measure of model validation is the ρ_A value, which should also exceed 0.7. To evaluate the distinctiveness of the constructs, the heterotrait-monotrait ratio of correlations (HTMT) is employed, while the variance inflation factor (VIF) is used to assess multicollinearity. For this PLS-SEM analysis, path model evaluation and bootstrap simulations (with 10,000 resamples) were conducted using SmartPLS 4.1.0.6 (48), a software program specifically designed for path model analysis and bootstrap simulations.

To assess the model's effectiveness, both the R^2 and the path coefficient must be considered. The R^2 quantifies the accuracy of the model's predictions by quantifying the extent to which the independent exogenous latent variables explain the variance in the endogenous latent variables. R^2 values range from 0 to 1, with different ranges indicating varying levels of predictive capability: a value between 0.25 and 0.49 indicates low predictive capability; a value between 0.50 and 0.74 suggests moderate predictive capability; and

a value between 0.75 and 1 indicates a strong predictive capability (24). In regression analysis, the path coefficient is equivalent to the regression coefficient. Direct relationships in the model are assessed by the corresponding path coefficients, while indirect relationships in the model are evaluated by multiplying the relevant path coefficients. This approach allows for a detailed understanding of both direct and indirect associations within the model.

Convergent validity is assessed using indicator reliability (IR) and AVE. Indicator reliability reflects the degree to which an indicator accurately captures its intended latent variable. According to Hair et al. (21), indicators with an IR value between 0.4 and 0.7 may be considered for removal if doing so improves the overall quality of the model. However, indicators with an IR value below 0.4 should always be excluded (29, 49). Despite these guidelines, the decision to remove indicators should be

approached with caution, as it is important to consider their potential contribution to the model's content validity (23). AVE is a statistical measure that quantifies the percentage of variance in an indicator that is attributable to its related latent construct. Generally, AVE values of 0.5 or higher are preferred, as they indicate satisfactory convergent validity.

Evaluation of the structural model. The quality of a structural model is primarily evaluated using two key criteria: path coefficients and R^2 . Path coefficients represent the standardized regression coefficients within the model, reflecting the strength and direction of relationships between constructs (30). These values range from -1 to 1 , with positive values indicating positive associations and negative values representing negative relationships. The magnitude of the coefficient reflects the strength of the connection, with values approaching $+1$ or -1 representing stronger correlations, while values near 0 indicate weaker, potentially insignificant associations (21). The statistical significance of path coefficients is typically assessed through bootstrapping techniques. The coefficients of determination measure the proportion of variance in an endogenous construct (dependent variable) that is explained by its associated antecedent constructs (independent variables) within the model (26). Higher R^2 values indicate greater explanatory power of the model for the endogenous construct. In the context of marketing research, R^2 values are generally interpreted as follows (26): weak: 0.25, moderate: 0.50, strong: 0.75.

Split-half scenario. To evaluate the accuracy of the SEM, a split-half consistency method was utilized (51). After conducting SEM on the original dataset, the data was divided into two equal parts ($n_1 = 63$, $n_2 = 63$) using the 'tidyverse' package (61) in the R programming language. SEM was then performed on each of these halves to evaluate the consistency and robustness of the model across different subsets of the data.

Results and discussion

The initial model was rigorously assessed against the measurement quality criteria detailed in the

Tab. 2. Descriptive of the initial SEM model

Related latent variable	Indicator	n	Mean	Median	Minimum	Maximum	Standard deviation
Biological stress (endogen)	TAS (Total antioxidant status)	126	1.437	1.410	0.682	2.230	0.363
	TOS (Total oxidant status)	126	8.022	7.722	3.289	15.703	2.744
	Hydrogen Peroxide	126	0.401	0.390	0.140	0.810	0.161
Biological variables (endogen)	WBC	126	17.296	16.300	4.720	39.100	5.365
	RBC	126	3.239	3.290	2.230	4.500	0.390
	HCT	126	19.814	19.600	14.000	29.300	2.634
	HGB	126	8.256	8.140	4.500	14.000	1.618
	MCV	126	60.719	60.557	57.600	65.500	1.419
	MCH	126	26.003	26.400	9.056	43.100	5.494
	MCHC	126	42.495	43.300	15.400	71.400	9.078
	PCT	126	0.009	0.008	-0.003	0.034	0.007
	MPV	126	12.377	12.324	2.901	26.100	5.304
	PDW	126	23.366	22.641	12.300	38.500	7.005
	PLT	126	5.946	5.819	1.120	14.900	2.737
	RDW	126	29.187	29.000	17.680	45.400	4.366
	Physical activity (endogen)	VLDL	126	32.596	29.000	3.000	91.000
LDL		126	40.286	39.000	18.000	71.000	12.290
HDL		126	113.967	114.000	80.000	159.794	16.380
Total Chol.		126	186.849	180.000	129.000	247.000	28.004
Triglycerides		126	162.981	145.000	15.000	455.000	74.838
Growth (endogen)	Total Protein	126	2.231	2.100	1.500	3.100	0.342
	Calcium	126	12.890	12.000	1.900	23.100	3.992
Physiological stress (endogen)	Cortisol	126	0.421	0.420	0.340	0.520	0.039
	CRP	126	0.015	0.010	0.010	0.030	0.007
Immun system (endogen)	IGG	126	0.693	0.480	0.280	1.540	0.417
	IGM	126	0.604	0.320	0.170	2.130	0.552
	IGA	126	0.386	0.330	0.210	0.770	0.138

‘Materials and methods’ section. To further validate this final model, it was applied to a separate dataset using the split-half approach. Table 2 presents a summary of the descriptive statistics for all indicators included in the initial model.

Initial SEM. The initial SEM analysis identified six associations among seven latent variables, comprising six endogenous latent variables (LOC) and one exogenous latent variable (HOC). Consequently, six arrows were identified, each linking the HOC to one of the six LOCs. These latent variables were represented by a total of 26 indicators (Fig. 2).

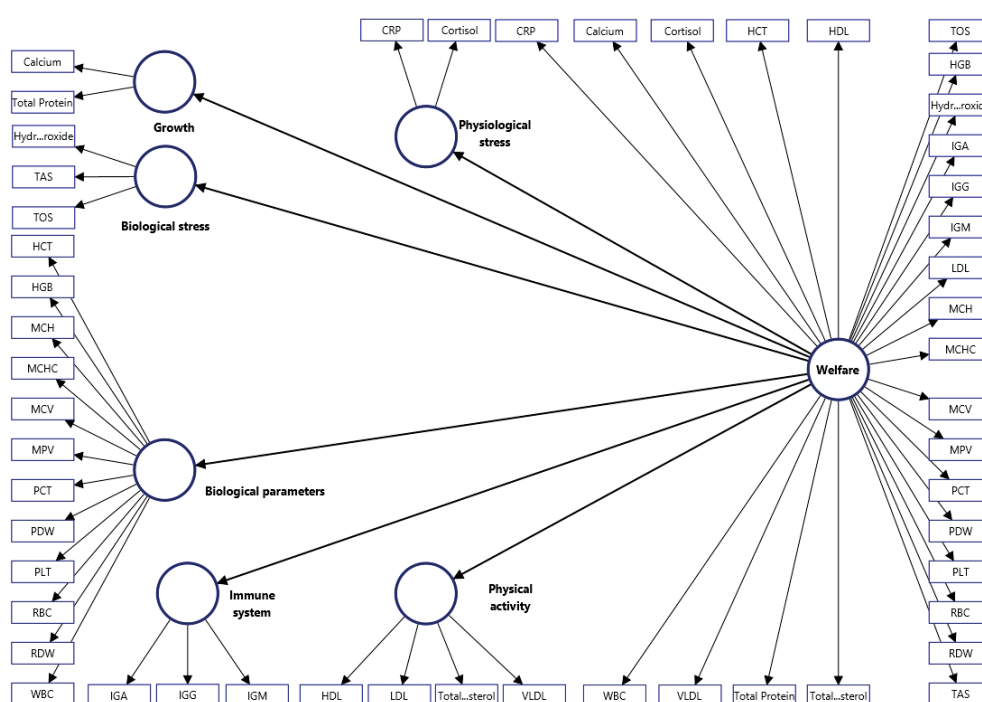


Fig. 2. Initial model for SEM

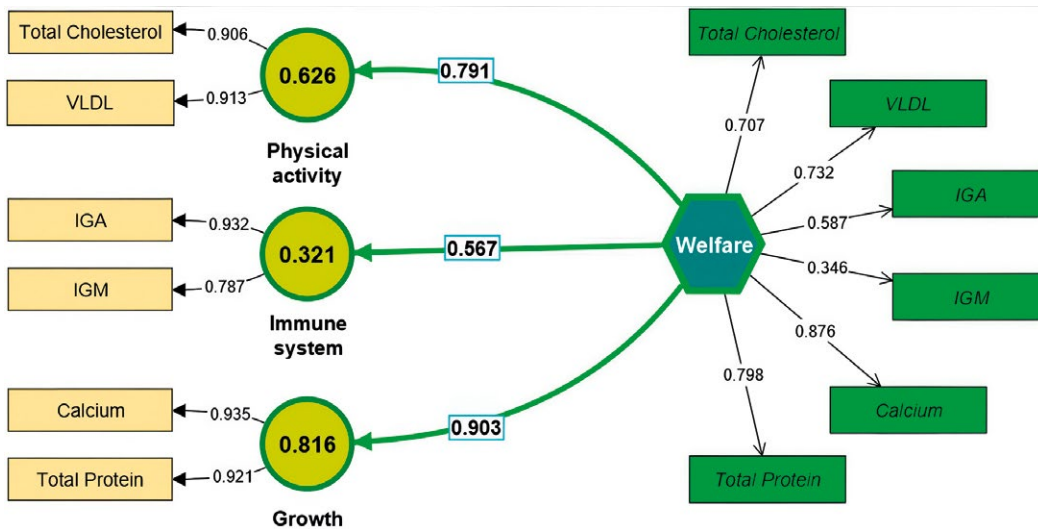


Fig. 3. Final SEM model

Final SEM. After conducting the final SEM, we identified three connections among four latent variables: three dependent variables (LOC) and one independent variable (HOC). Based on the quality criteria for indicator reliability, 20 out of the initial 26 indicators were removed from the model because their loadings were below 0.4 or they did not attain statistical significance. Only statistically significant indicators were retained in the model (21). Additionally, among the endogenous measurement latent variables, biological stress (indicators: TAS (total antioxidant status), TOS (total oxidant status), hydrogen peroxide) and physiological stress (indicators: CRP, Cortisol) were excluded from the model due to their negative impact on the predictive validity of the SEM (21) (Fig. 3).

Evaluation of Measurement Models (Final SEM). The reliability and validity of the measurement models in SEM were evaluated using the CR, IR, and AVE criteria as outlined in the ‘Material and methods’ section. Following the initial application of the Partial

Least Squares Structural Equation Modeling (PLS-SEM) technique, all indicators with loadings below 0.4 or lacking statistical significance were removed from the original model. Only indicators that demonstrated statistical significance were retained (21). For physical activity, the indicators VLDL (0.913) and total cholesterol (0.906) showed similar indicator reliability, indicating that both predict the endogenous latent variable

physical activity equally well. In the case of the immune system, the indicator IgA (0.932) showed higher indicator reliability than IgM (0.787). Although both indicators effectively predict the endogenous latent variable immune system, the IgA indicator provides a more accurate prediction than IgM.

The indicator calcium (0.935) accurately predicts the growth status of Japanese quail, achieving a similar level of accuracy as the indicator total protein (0.921). Among the indicators used to assess the exogenous latent variable of well-being, calcium (0.876) was the most accurate, followed by VLDL (0.732), total protein (0.707), total cholesterol (0.707), IgA (0.587), and IgM (0.346) (Tab. 3). We then evaluated the reliability of the measurement models using the CR method. The immune system model had the lowest reliability (CR = 0.852) among the measurement models, while the growth and physical activity models showed the highest reliability (CR = 0.926 and CR = 0.905, respectively). The composite reliability of the welfare

Tab. 3. Indicator reliabilities (IR), coefficients of determination (R²), composite reliabilities (CR), average variance explained (AVE), and effect size (f²) values for the final SEM, halved consistency SEM₁ and halved consistency SEM₂

		Final SEM					Split-half consistency SEM ₁					Split-half consistency SEM ₂				
		IR	CR	AVE	R ²	f ²	IR	CR	AVE	R ²	f ²	IR	CR	AVE	R ²	f ²
Growth (endogen)	Calcium	0.935	0.926	0.862	0.82	4.44	0.93	0.92	0.85	0.83	4.71	0.94	0.94	0.88	0.81	4.39
	Total Protein	0.921					0.91					0.94				
Immune system (endogen)	IGA	0.932	0.852	0.744	0.32	0.47	0.98	0.87	0.77	0.17	0.21	0.88	0.80	0.67	0.51	1.03
	IGM	0.787					0.77					0.76				
Physical activity (endogen)	Total Choles.	0.906	0.905	0.827	0.63	1.68	0.92	0.92	0.84	0.68	2.09	0.88	0.90	0.81	0.64	1.80
	VLDL	0.913					0.92					0.92				
Welfare (exogen)	Calcium	0.876	0.841	0.484	-	-	0.88	0.82	0.46	-	-	0.85	0.87	0.53	-	-
	IGA	0.587					0.46					0.66				
	IGM	0.346					0.16					0.49				
	Total Choles.	0.707					0.76					0.66				
	Total Protein	0.798					0.78					0.84				
	VLDL	0.732					0.75					0.78				

status of Japanese quail was found to be 0.841, which falls within the acceptable range. Therefore, all measurement models were considered reliable, as their composite reliability exceeded the threshold of 0.6 (Tab. 3). The validity of the measurement models was evaluated using the AVE test criteria. All measurement models, including the welfare status of Japanese quail (physical activity AVE = 0.827), had AVE values demonstrating validity. The AVE for the immune system was 0.744, and for growth was 0.862. All AVE values are well above the threshold of 0.5, providing strong support for the validity of the measurement models (30). The lowest calculated AVE value for the welfare level of Japanese quails is 0.484, which is very close to the threshold value, thus supporting the validity of the models (Tab. 3).

Evaluation of the Structural Model (Final SEM).

The structural model was evaluated, revealing all links between exogenous and endogenous latent variables were statistically significant ($p < 0.05$). The welfare condition of Japanese quail had a substantial influence on their growth, as indicated by a path coefficient of 0.903. Additionally, the welfare level of Japanese quail had a more pronounced effect on their physical activity ($P_1 = 0.791$) than on their immune system ($P_2 = 0.567$). The R^2 values for the structural model were calculated as follows: 0.816 for growth, 0.626 for physical activity, and 0.321 for the immune system. This means that the model explained 81.6% of the variation in growth, 62.6% of the variation in physical activity, and 32.1% of the variation in the immune system. Therefore, the structural model accounts for the greatest degree of variation in growth, followed by physical activity and finally, the immune system (Tab. 2).

Evaluation of split-half scenario PLS-SEM. The reliability and validity of the model were assessed through cross-validation using split-half scenarios, designated as SEM1 and SEM2. The test criteria of the SEMs exhibited patterns comparable to those observed in the final SEM (Tab. 2). Furthermore, the correlations (path coefficients) between the inner and outer latent variables were found to be statistically significant, with a significance threshold of $p < 0.05$. This further confirms the robustness of the model across different data subsets.

Assessment of the measurement models. Among the original 26 indicators, six were identified as reliable for estimating the latent variables of interest. The indicators of endogenous latent variables exhibited high loadings (above 0.7), confirming their suitability for assessing these latent variables. In terms of the reliability of the indicators for the exogenous latent variable, quail welfare, the factors 'Physical activity', 'Immune system' and 'Growth' all showed reliability values above 0.7. However, the indicator IGM, with a reliability below 0.4, was not excluded from the model. This decision aligns with the principle that indicators with reliability between 0.4 and 0.7 should only

be excluded if doing so enhances the model's quality criteria (24). Moreover, as in this study, the removal of indicators should be approached with caution if they contribute to content validity (22). Among the indicators, calcium showed the highest reliability, suggesting it is the most appropriate indicator for measuring the growth latent variable. This was followed by IGA, Total protein, VLDL, Total cholesterol and IGM. This ranking underscores the importance of these indicators in accurately assessing the relevant latent variables.

These results seem realistic, as cholesterol and its derivative, VLDL, proved to be better indicators of the physical activity levels of the quails studied. Other studies have similarly found that physical activity increases when animals are provided with ample space (15), leading to an increase in HDL levels, and a corresponding decrease in VLDL and total cholesterol levels. This pattern is often observed when animals are in more favorable conditions, such as environments that minimize threats like leg disorders (47, 56) or provide favorable environmental conditions (54).

The suitability of the „IgA” indicator for estimating the immune system is likely due to its role as an immunoglobulin that provides the first line of defense on mucosal surfaces, preventing pathogens from binding and spreading within the body (8). Additionally, IgA has been reported to contribute to immunomodulatory activity (63). Beyond its role in immune exclusion – a nonspecific intestinal function – IgA supports the maintenance of appropriate bacterial communities in specific intestinal compartments (58) playing an important role in specific immunity and immune regulation (37). Moreover, IgA displays a range of antibody functions that enhance the protective capacity of the intestinal mucosa and inhibit viral replication (9). Therefore, IgA serves as an important indicator of poultry health. Besides, the presence of IgA and IgM probably indicates increased welfare, as they are critical components of basic health and biological functioning, which are essential aspects of animal welfare. This underscores the appropriateness of using IgA to predict the immune system of Japanese quail (40).

IgM showed lower indicator reliability compared to IgA, which may be attributed to the differing roles of these immunoglobulins as indicators of various conditions. Immunoglobulin M (IgM) is crucial in the immune system's response to pathogens, particularly through its activation of the complement system, which is responsible for destroying bacteria, viruses, and other foreign substances (38). The variability in IgM's performance as an indicator could be due to its role being more context-specific, as opposed to the broader, more consistent function of IgA in mucosal immunity and immune regulation. This difference might explain why IgM did not perform as reliably as IgA in this study.

However, the IgM indicator showed lower indicator reliability than IgA. This may be attributed to the

differing roles of IgA and IgM as indicators under various conditions. IgM participates in immune functions such as lysing bacteria and viruses by activating the complement system (38). The lower reliability of IgM in predicting certain aspects of the immune system could be related to the broad range of immune functions it performs, which may vary significantly depending on the context. This diversity in function might contribute to its less consistent performance as an indicator compared to IgA.

IgM is the first antibody produced and binds to antigens, acting as the initial line of defence (31), while IgA is the antibody primarily responsible for protecting against antigens on mucosal surfaces, where it binds extremely well (55). The primary antibody response provided by IgM offers a broad but less specific defense (1). In contrast, IgA, which is abundant on mucosal surfaces, provides a specific defense against pathogens. This difference in specificity may limit efficacy of IgM compared to IgA (42).

The role of IgM in limiting gut microbes also differs from that of IgA, with IgM can only modestly compensating for the absence of gut IgA (44). Furthermore, abnormalities in IgM are associated with treatment-resistant immune thrombocytopenia (1). Therefore, the greater impact of IgA on the immune system compared to IgM, can likely be attributed to its specificity and its strategic location on mucosal surfaces, where it plays a critical role in immune defence.

Overall, all reliability values exceeded 0.7, indicating satisfactory combined reliability for the model (30). Therefore, it can be concluded that the four latent variables were adequately measured by the respective indicators. Three of the measurement models exhibited AVE values above the recommended threshold of 0.5 proposed by Henseler et al. (30). However, the AVE for the exogenous latent variable welfare structure was found to be 0.48, which is slightly below the recommended threshold. The lower AVE value may be attributed to the relatively low indicator reliabilities of IgA (IR = 0.587) and IgM (IR = 0.346). The lower AVE suggests that the welfare construct may not be fully captured by these indicators, potentially leading to an underestimation of the entire exogenous latent variable. Nevertheless, the study represents a pioneering effort in establishing a framework for measuring and understanding the welfare status of quails. As research progresses, it is expected that more specific and reliable indicators will be identified. In conclusion, while the current indicators provide a valuable starting point, additional indicators are needed to estimate the welfare status of quail more accurately and comprehensively.

Assessment of the structural model. The relationships between the endogenous and exogenous variables were found to be statistically significant when analyzed using the bootstrap method. Furthermore, the HTMT (Heterotrait-Monotrait) values of the latent variables were all below 0.85, indicating that each construct was

distinct and represented by its own set of indicators. This supports the conclusion that the welfare status of the quails had a significant influence on the three final variables. Among these relationships, the highest path coefficient between the quails' welfare status and growth ($P_3 = 0.903$), indicating growth was the most significantly influenced variable, followed by physical activity ($P_1 = 0.791$) and the immune system ($P_2 = 0.567$). These findings align with existing literature, which suggests that growth is a strong indicator of good welfare in birds (2, 45).

The analysis estimated that an improvement in quail welfare positively affects growth, with a unit increase in welfare leading to a 0.901-unit increase in growth. Furthermore, a unit increase in welfare was associated with a 0.567-unit increase in the immune system. Moreover, physical activity was shown to positively influence productivity, with a 0.791-unit increase resulting from enhanced welfare. These results underscore the significant impact of welfare on various aspects of quail health and development.

These results also suggest that physical activity, immune system function, and growth may serve as effective indicators for a realistic assessment of the welfare status of quails. Consequently, it can be inferred that the other variables excluded from the quality assessment of the SEM are not significantly influenced by the welfare status of quails and, therefore, are not suitable for capturing their welfare status in practical applications.

The model exhibited a moderate R^2 value for physical activity (62.6%), a weak R^2 value for the immune system (32.1%), and a high R^2 value for growth (81.6%). This suggests that the welfare status of quails explains the variance in growth quite well, moderately well for physical activity, and to a lesser extent for the immune system. However, further investigation is required to ascertain whether these findings are consistent across different contexts and to enhance our understanding of emotional state of quails and other animals, it would be beneficial to evaluate and explore other indicators across species.

Comparison of the final SEM with the split-half scenario SEM. The validity and reliability values of the SEM scenarios in the split-half analysis are comparable to those of the final SEM model, indicating that the final model exhibits an adequate level of model quality (Tab. 3). Consequently, the final SEM model serves as a robust framework for testing research hypotheses, making informed decisions, and guiding future studies.

Concluding statement. The results indicate that the welfare of Japanese quail significantly influences the examined latent variables, with effect sizes ranging from moderate to high. These findings indicate that the model's outputs are suitable for practical applications. This study employed Partial Least Squares Structural Equation Modeling (PLS-SEM) and represents one

of the first attempts to estimate welfare in Japanese quail, demonstrating the feasibility and potential of this approach.

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Corresponding author: Mehmet Saraçoğlu, PhD Candidate; e-mail: mehmetсароглу@harran.edu.pl