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Research Article/Araştırma Makalesi

The assessment of poultry welfare using partial least squares-path modeling (PLS-SEM): a modeling the effects of physical activity and stress on growth

Kısmi en küçük kareler-yol modellemesi (VT-YEM) kullanılarak kanatlı refahının değerlendirilmesi: fiziksel aktivite ve stresin büyüme üzerindeki etkilerinin modellenmesi

Hamza YALÇIN[1], Zeki DOĞAN[1], Sedat ŞEN[2], Mehmet ÇETİN[1], Mehmet GÖÇMEN[1], Abdurrahim KOÇYİĞİT[3]

[1]Harran Üniversitesi, Ziraat Fakültesi, Zootekni Bölümü, Şanlıurfa
[2]Harran Üniversitesi, Eğitim Fakültesi, Eğitim Bilimleri Bölümü, Şanlıurfa
[3]Bezmialem Vakıf Üniversitesi, Tıp Fakültesi, Temel Tıp Bilimleri Bölümü, Şanlıurfa

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Address for Correspondence:
Hamza YALÇIN
e-mail:
hyalcin@harran.edu.tr

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ABSTRACT

Poultry welfare is closely related to human health and animal production which is a sensitive process. While several statistical methods are available to measure observed variables (stocking density), unobserved (latent) variables such as emotional, psychological situations are typically analyzed using factor analysis. In recent years, structural equation modeling (SEM) has been used effectively in many fields such as agriculture and livestock. SEM provides an estimation of relationships between latent variables that cannot be measured directly. Most of the studies published on poultry welfare highlighted the need to determine causal relationships between latent variables (growth, physical activity, biological stress). The purpose of this study was to examine the relationship between biological stress, physical activity, and growth in livestock using PLS-SEM analysis. A three-factor model was conducted with 96 animals. The PLS-SEM results revealed that 65.2% of growth can be predicted by the independent variables (R^2 = 0.652), and that physical activity (γ = 0.698) was found to have more effect than biological stress (γ = 0.176) on growth. Also, physical activity was determined as the main variable for the evaluation of biological stress (γ = 0.546, R^2 = 0.298) and growth. Overall, the research showed the suitability of SEM for the assessment of growth and biological stress.

Key Words: Growth, Physical activity, PLS-SEM, Poultry, Welfare

ÖZ

Kanatlı refahı, insan sağlığı ve hassas bir süreç olan hayvan üretimi ile yakından ilgilidir. Gözlemlenen değişkenleri (yerleşim sıklığı) ölçmek için çeşitli istatistiksel yöntemler mevcut olsa da duygusal, psikolojik durumlar gibi doğrudan gözlemlenmemiş (gizil) değişkenler tipik olarak faktör analizi kullanılarak analiz edilir. Son yıllarda yapısal eşitlik modellemesi (YEM) tarım ve hayvancılık gibi birçok alanda etkin bir şekilde kullanılmaktadır. YEM, doğrudan ölçülemeyen gizli değişkenler arasındaki ilişkilerin bir tahminini sağlar. Kanatlı hayvan refahı üzerine yayınlanan çalışmaların çoğu, gizil değişkenler (büyüme, fiziksel aktivite, biyolojik stres) arasındaki nedensel ilişkileri belirleme ihtiyacını vurgulamaktadır. Bu çalışmanın amacı, PLS-SEM analizi kullanılarak çiftlik hayvanlarında biyolojik stres, fiziksel aktivite ve büyüme arasındaki ilişkiyi incelemektir. Çalışmada, 96 hayvanın bulunduğu üç faktörlü bir model oluşturulmuştur. VT-YEM sonuçları, büyümenin %65.2'sinin bağımsız değişkenler (R^2 = 0.652) tarafından tahmin edilebildiğini ve fiziksel aktivitenin (γ = 0.698) büyüme üzerinde biyolojik stresten (γ = 0.176) daha fazla etkiye sahip olduğunu ortaya koymuştur. Ayrıca biyolojik stres (γ = 0.546, R^2 = 0.298) ve büyümenin değerlendirilmesinde fiziksel aktivite ana değişken olarak belirlenmiştir. Genel olarak araştırma, YEM'in büyüme ve biyolojik stresin değerlendirilmesi için uygunluğunu göstermiştir.

Anahtar Kelimeler: Büyüme, fiziksel aktivite, VT-YEM, kümes hayvanları, refah

Introduction

Animal farming is a sensitive process. It seems that modern society is concerned about food production processes/systems. When discussing animal welfare, the restriction of the movement of animals, their rapid growth, high production rates, and merciless practices attract the attention of the general public (Ingenbleek et al., 2012). The health of the animals is an undisputed need, and health is the basic requirement for animal welfare because worse health causes less welfare (Ma et al., 2014). Nowadays, the increase and high price of meat consumption lead to a different product, such as poultry, which is more commercially effective (Gholami et al., 2020). The costs of feeding methods in poultry have an important share. For example, the cost of ration in poultry can range from 55% to 75% of the total production cost. However, some factors (genetic, nutritional, and environmental conditions) may play a significant role in making poultry more efficient, such as lowering feed costs (Kryeziu et al., 2018). Broiler meat production has experienced a significant change in terms of nutrition, genetics, and management, and increased yield in the past 50 years. Therefore, broiler chickens are reported to have the highest yield among other meat-producing animals (Costantino et al., 2018).

There are many factors that affect broiler chicken growth such as stress, physical activity, immune system, and so on. These factors cannot be measured directly but just can be indirectly assessed by some other indicators. For instance, the physical activity of chickens affects some serum parameters as total cholesterol, high-density lipoprotein (HDL), and very-low-density lipoprotein (VLDL) (Park et al., 2018; Wu et al., 2018). From a different viewpoint, measurements like the total peroxide, total oxidant status (TOS), and total antioxidant status (TAS) are used at the evaluation of biological stress (Lykkesfeldt & Svendsen, 2007). In addition to these poultry growth is affected by total protein and calcium levels also (Tesseraud et al., 2011; Fang et al., 2017).

Rearing in good conditions of broilers is a good approach to decrease stress, pain, diseases, and to increase health, activity, and welfare. Modern broiler chickens are raised for rapid growth, and studies have indicated that they spend about 80% of their lifetime to rest (Weeks et al., 2000). However, combined with immobility and fast growth, it creates a risk factor for the evolution of lameness and pathologies associated with the locomotor system (Bradshaw et al., 2002) and is likely to result in less welfare due to pain for the chickens and commercial losses for the producer. This increases stress and affects the yield negatively (Pedersen et al., 2020).

Oxidation, which occurs as a result of metabolic events, initiates the formation of some damage by creating free radicals in the organism. Compounds that struggled with these damages have been emphasized (Wei & Pang, 2005). The balance between the production of free radicals and antioxidant defence is important for health. The level of free radicals, that is, being too little or too much, can cause serious damage such as temporary or permanent. Many mineral substances form powerful antioxidants against the damage of free radicals produced by the organism (Anonymous, 2018).

Pointed out clearly in some studies in which growth, in broiler production, is influenced by factors such as genetic, feed type, feed conversion rate, lighting programs, litter and housing type, live weight gain, and viability (Mello et al., 2012). Poultry requirements for phosphorus are influenced by many factors, including the calcium level (Rama Rao et al., 1999). Phosphorus and calcium, in many processes for animal biology, are necessary minerals. Minerals of P and Ca that stored in the skeleton, are founded plentiful in animals respectively 80% and 99%. Besides, these minerals play a crucial role not only in bone development but also in mineralization (Proszkowiec-Weglarz & Angel, 2013). In addition, protein and amino acids play an important role in achieving chicks' balanced diet and success in rapid growth (Beski et al., 2015).

The many ways of evaluating animal welfare measurement, have different advantages over each other (Mench, 2018). There are many studies on agriculture (Villeneuve et al., 2018) and animal science (Drews et al., 2018), however, previous studies have not yet presented any suggestions on the use of multivariate modeling, specifically SEM, in the context of poultry-based welfare science (Collins & Part, 2013). Collins and Part (2013) noted that, "mathematical modelling could assist us in identifying, and tackling, any existing or impending conflicts between, for example, farm animal welfare, farmer livelihood, future food security and environmental legislation", without providing any approach on a clear choice by using welfare modeling.

The current study addressing this research gap, investigates the conditions supporting the use of each latent variable and the respective measurement model when establishing animal welfare modeling in PLS-SEM. There are many factors that can be measured directly and indirectly which are called indicator variables and latent variables, respectively. PLS-SEM enable the analysis of the relationship between observed and latent variables together. Although PLS-SEM is used commonly in marketing and social science with the increasing popularity from day to day (Hair et al., 2020). Interest in SEM by researchers has also increased about agricultural issues (Villeneuve et al., 2018) for instance, agricultural research (Doğan et al., 2021), animal welfare (Krugmann et al., 2020) and, milk production (Heringstad et al., 2009). The purpose of this study was to examine the relationship between biological stress, physical activity, and growth in livestock using PLS-SEM analysis.

The model is defined based on the literature. The concept and their synthesis is shown in Figure 1.

H1. Physical activity is related to biological stress.
H2. Biological stress is related to growth.
H3. Physical activity is related to growth.

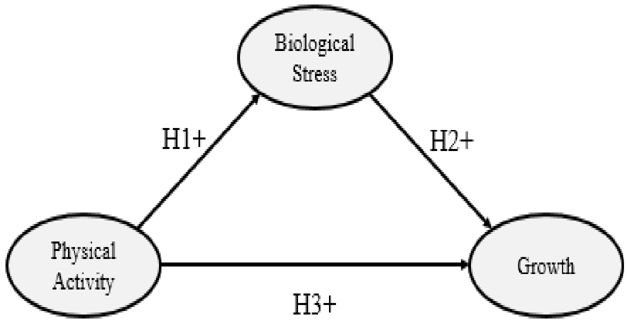

Figure 1 Theoretical model and hypothesis

Materials and Methods

Material

The model in the present study was established with the data obtained from two projects numbered 12078 and 13110, which were carried out under the same conditions and supported by Harran University Scientific Research Commission (HÜBAK) in 2013. In these projects, trials were established with Ross 300 broiler chickens. Two different studies (Çetin & Göçmen, 2015; Çetin et al., 2019) were conducted with some data on these projects. In these experiments, a total of 96 broiler chickens was used. The following parameters are used in this study: total oxidant status (TOS), total antioxidant status (TAS), total protein, calcium (Ca), total cholesterol, high-density lipoprotein (HDL), and very-low density lipoprotein (VLDL) (was determined according to the Friedewald equation (Friedewald et al., 1972).

Method

PLS-SEM modeling

The PLS-SEM, which is a member of the structural equation modeling (SEM) family, was developed by Wold (1982) as a "soft modeling" compare to covariance-based structural equation modeling (CB-SEM) (Hair et al., 2017). The PLS-SEM is frequently called "variance-based SEM", and it is also a nonparametric technique. PLS-SEM, using multiple indicators, estimates complex relationships between latent variables that are endogenous (dependent- η) and exogenous (independent- ξ) variables. The latent variables are described by path coefficients (γ) pointing to each other.

The PLS-SEM is composed of two models: 1) measurement model (called the outer model) which examines the relationship between latent variables and their indicator variables, and 2) structural model (called the inner model) which examines relationships between latent variables. In the structural model, relationships between latent variables are indicated by arrows from exogenous to endogenous. A PLS path model is estimated at 2 stages: the outer models are examined in stage 1, and the inner model is examined in stage 2. The first stage is estimated iteratively, but not the second stage (Hair et al., 2017).

There are two approaches to measure for the outer model, as a formative measurement model and a reflective measurement model. The one is the formative measurement model, where causality is from indicators to the relevant latent variable (η or ξ). So, formative measurement models are caused by one or more indicators and the direction of the arrows are connecting from indicators to the structure. In the reflective measurement model, the direction of the arrows is pointing from the construct to the indicator variables that were caused by the construct. Also, each indicator has an error term. It is assumed that the error terms are not valid for formative measurement (Esposito Vinzi et al., 2010).

The endogenous variables, in an SEM model, are affected by the exogenous variables or other endogenous variables. But the exogenous variables are not affected by any other latent variables. Finally, when considered from this point of view, as in linear regression analysis, the coefficient of determination (R^2) is only calculated for a dependent, in other words, the endogenous variables, but not for exogenous (independent) variables. The latent variables and their indicator variables used in this study are given in Table 1.
In this experimental study, the SmartPLS (v.3.2.9) (Ringle et al., 2015) was used to analyse and test the hypothesis using the PLS-SEM technique. The assessment of model results was made as recommended by Hair et al. (2019). In the PLS-SEM, there are two types of evaluations: 1) the measurement model (each evaluated separately, the reflective and the formative), and 2) the structural model.

Table 1. Description of the latent variables (η or ξ), their measurement models, and associated measurement variables.

Latent variables	Measurement (indicator) variables
Physical activity ξ_1 (Reflective mode)	Total Cholesterol High-density lipoprotein (HDL) Very low-density lipoprotein (VLDL)
Oxidative stress η_1 (Formative mode)	Total antioxidant status Total oxidant status Total peroxide
Growth η_2 (Formative mode)	Calcium (Ca) Total protein

The assessment of the reflective measurement models

The indicator loadings should be ⩾ 0.708 (acceptable reliability of indicators); internal consistency reliability (as using composite reliability-CR) should be ⩾ 0.70; the average variance extracted (AVE) used to evaluate the convergent validity that explains the variance of measurement variables of the structure should be ⩾0.50. As the latest criteria, the cross-loadings suggest that to testing discriminant validity for latent variable' indicators. They indicate that the correlation of the indicators with its structure and should be higher than its correlation with other structures in the model (Hair et al., 2019).

The assessment of the formative measurement models

Indicator collinearity (variance inflation factor-VIF) should be <5; for weights of the indicator should be examined in terms of statistical significance and relevance, according to bootstrapping procedure (Hair et al., 2019).

The assessment structural (inner) models

The coefficient of determination (R^2) is referred to as the explained variance on each of the endogenous constructs of all the other constructs (endogenous or exogenous). R^2 values range from 0 to 1 and are classified as ⩾0.75-substantial, 0.75 - 0.50-moderate, and 0.50 - 0.25-weak. The effect sizes (f^2) are described as 0.02-

small, 0.02-0.15-medium, and ⩾0.35-large (Hair et al., 2019).

Lastly, when evaluating the significance ($p<0.05$) of the parameter estimates of path coefficients, outer loadings, outer weights are assessed using a nonparametric bootstrapping procedure. Bootstrap, recommended by Cheah et al. (2018) as 5000 sub-sample to derive standard errors to evaluate parameter estimates and, to assess the significance of each estimate, the critical t-value must be higher than 1.65 (according to one-tailed test).

Results and Discussion

SmartPLS reports indicator weights/loadings, CR, AVE, path coefficients, fornell-larcker criterion, cross-loading, heterotrait-monotrait ratio of correlations (HTMT), VIF (for both indicators and structures), t-value and p-value. Three-structure/eight-indicator models, as viewed in Figure 1 and 2, were established and analyzed with the SmartPLS.

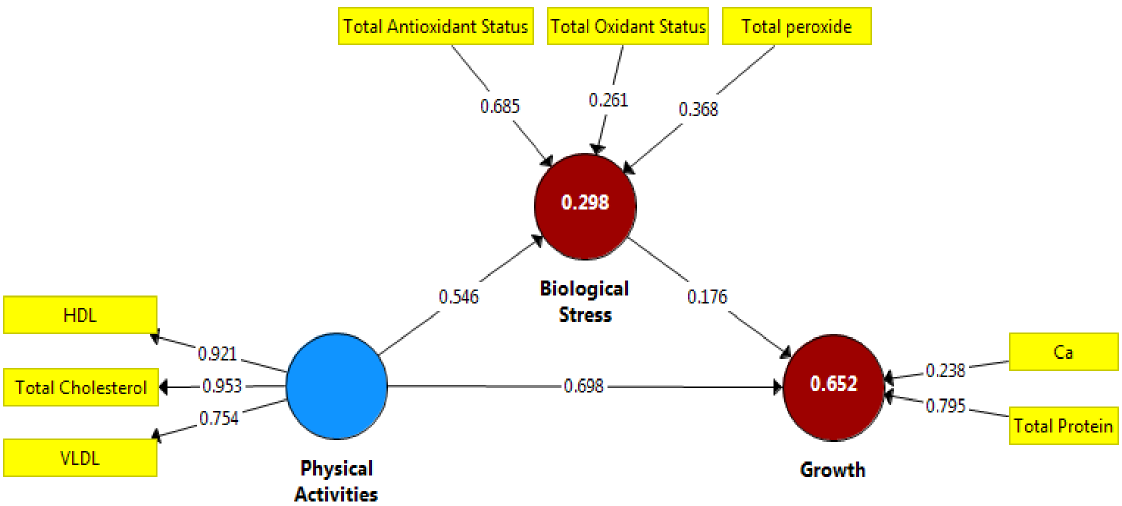

Figure 2. Results of PLS-SEM.

Assessing of the measurement models

There are two types of measurement models, which reflective measurement model (Table 1) and the formative measurement model (Table 2), in this study. The first step in evaluating a PLS-SEM model is to examine the outer model in an effort to validate the measurement model (Hair et al., 2019). To do this, firstly, the relationships between structures and their indicators were examined. The physical activity is a 3-indicator structure. The results of the physical activity were the value of AVE, CR, CA, and Rho_A, which were observed as 0.774, 0.911, 0.851, 0.902, as shown in Table 2, respectively. It was seen that the cross-loading criteria were met for discriminant validity (Table 2). The construct of the physical activity appeared to meet the criteria according to Hair et al. (2017).The contribution of the total cholesterol on the construct was found higher than HDL and VLDL. Therefore, total cholesterol had the highest loading (0.953) and reliability (0.908) compare to HDL (0.921-0.848) and VLDL (0.754-0.569). This result indicated that the total cholesterol was the best estimates for physical activity. All three indicators of the physical activity were found to be significant based on bootstrapping procedure ($p<0.05$).

Table 2. Assessment of construct of Physical Activity (reflective measurement model)

	Reflective Indicators	Convergent Validity			Internal Consistency Reliability			Discriminant Validity	95% bootstrap confidence interval	t Value	Significant (p<0.05)?
		Loadings	Indicator Reliability	AVE	Composite Reliability	Cronbach's Alpha	Rho_A				
		>0.7	>0.5	>0.5	0.70-0.95						
Physical Activity	TC	0.953	0.908						[0.933, 0.963]	113.81	**Yes**
	HDL	0.921	0.848	0.774	0.911	0.851	0.902	**Yes**	[0.886, 0.941]	57.27	**Yes**
	VLDL	0.754	0.569						[0.629, 0.835]	12.19	**Yes**

TC: Total cholesterol, HDL: High-density lipoprotein, VLDL: Very low-density lipoprotein

The latent variables of growth and biological stress are established as a formative measurement model (Table 3). There was no multicollinearity among all indicators (the VIF values ranging from 1.11 to 3.1 and the tolerance value ranging from 0.32 to 0.90). For the growth, the weight of total protein (w=0.795) affected the latent variable more than Ca (w=0.238) measurement. Total protein and Ca indicators were found to be significant ($p<0.05$). The biological stress was best estimated by the total antioxidant status (w=0.685) compare to the total peroxide (w=0.368) and particularly the total oxidant status (w=0.261). The effects of the total antioxidant status with respect to the results of the bootstrap procedure were significant on the biological stress variable but not the total oxidant status and the total peroxide.

Table 3. Assessments of constructs of growth and Biological Stress (formative measurement models)

	Formative Indicators	Outer Weights (Outer Loadings)	t Value	Significance (p<0.05)?	95% bootstrap confidence interval	VIF (Tolerance Value)
Growth	Calcium	0.238 (0.892)	1.933	**Yes**	[0.072, 0.487]	3.101 (0.322)
	Total Protein	0.795 (0.991)	6.889	**Yes**	[0.548, 0.941]	3.101 (0.322)
Biological Stress	TAS	0.685 (0.852)	5.633	**Yes**	[0.519, 0.865]	1.112 (0.899)
	TOS	0.261 (0.584)	0.71	No	[-0.023, 1.034]	1.351 (0.740)
	TP	0.368 (0.717)	1.015	No	[-0.362, 0.683]	1.441 (0.694)

TAS: Total antioxidant status, TOS: Total oxidant status, TP: Total Peroxide

The assessment of the structural (inner) models

Discriminant validity is established when the correlation of an indicator with the structure to which it is assigned has a higher correlation than cross-loading with other structures. Thus, as shown in Table 4, discriminant validity between structures was provided with the PLS-SEM analysis.

Table 4. PLS-SEM cross-loadings test for discriminant validity

Indicators	Physical Activities	Biological Stress	Growth
Total Cholesterol	**0.953**	0.527	0.876
HDL	**0.921**	0.456	0.661
VLDL	**0.754**	0.457	0.500
TAS	0.493	**0.852**	0.449
TOS	0.246	**0.584**	0.396
Total Peroxide	0.392	**0.717**	0.398
Ca	0.715	0.473	**0.892**
Total Protein	0.785	0.560	**0.991**

HDL: High-density lipoprotein, VLDL: Very low-density lipoprotein

After the assumptions of reliability and validity of the constructs are established, structural model results are evaluated to define the relationships between variables. The multicollinearity between variables was examined before evaluating the structural model since path coefficients are estimated according to OLS regression. When the relationship between the model's constructs was examined, multicollinearity was found negligible (for both PA and BS was 1.425). The path coefficients' size and significance and the R^2 values have an important role in the assessment of the model. The path coefficients' sizes and level of significance, which represent hypothesized relationships between constructs, were evaluated.

Table 5. Assessment of path coefficients of the structural model

Constructs	Path coefficients	t Values	Significant (p<0.05)?	95% bootstrap confidence interval	f^2 Value (effect size)
PA → BS	0.546	8.353	**Yes**	[0.402, 0.630]	0.43 (large
BS → GR	0.176	1.350	No	[-0.002, 0.389]	0.06 (small)
PA → GR	0.698	7.689	**Yes**	[0.565, 0.844]	0.98(large)

PA: physical activity, BS: biological stress, GR: growth

Evaluation of the inner (structural) model was implemented in step 2 (Figure 2). There are 3 constructs and their 3 path coefficients as the model. Physical activity was the most significant variable for not only the biological stress but also the growth. Physical activity had a more key role in the growth (γ=0.698, p<0.05) than for the biological stress (γ =0.176, p>0.05).

Physical activity accounted for 29.8 % (R^2=0.298) of the total variability in biological stress according to the model, and this was a moderate effect. Also, physical activity and biological stress accounted for 65.2 % (R^2=0.652) of variation in the broiler growth (Figure 2). This coefficient of determination (R^2) was substantial. The effect sizes (f) of constructs were found to be significant as demonstrated in Table 5.

Assessment of measurement models

In the model of the current study, both the reflective measurement model and the formative measurement model were used. In the formative measurement models, VIF values (<3), weights, and significance of indicators were considered appropriate. For the reflective measurement model, indicators reliability and validity were considered appropriate for the estimation of the relevant latent variable. The lowest load of the indicators of the structure was found to be 0.83 and passed the 0.70 thresholds very well. Based on the literature and in accordance with the aforementioned criteria, all indicators used in the model seem to be most appropriate to measure the latent variables.

Measurement results of the current study seem to be practically suitable due to estimates and significances of the model created based on the literature. There is some evidence that physical activity causes significant changes in total cholesterol, HDL, and VLDL levels (Huttunen, 1982; Stucchi et al., 1991; Dawkins et al., 2004; Simsek et al., 2009). The fact that indicator of total cholesterol seems to be the most suitable indicator for estimating the physical activity in terms of reliabilities, could be related to the fact that the conducting physical exercise reduces total cholesterol and VLDL and increases HDL (Mann et al., 2014), resulting in improved body health. On the other hand, biological stress was affected by total antioxidant status much more, total oxidant status, and total peroxide, and this situation was a desired situation in terms of body health. The increase in TAS level possibly points to increased welfare due to the prevention of oxidative damage in the body (Celi & Gabai, 2015). The related factors, represented by serum total protein and calcium, contributed to growth in the model. This result was expected, because total protein, and calcium are generally closely related to their effect on growth. The effect of total protein on growth was estimated to be greater than calcium because, while the proteins are found in all muscles of the body (Tesseraud et al., 2011), calcium is essential for skeletal and bone development (Swennen et al., 2007). Also, low calcium intake while the organism is growing can result in sub-optimal bone mass and slowing growth (Swennen et al., 2007). Conclusively, the discriminant criteria of the established model showed that related constructs are appropriate for analysis of the model of animal welfare and can be interpreted according to literature, as mentioned above.

Assessment of structural model

Physical activity plays a significant role in the growth of living organisms such as adipose, bone, and muscle tissue (Alves & Alves, 2019). The fact that physical activity is less or more than optimum determines the direction of biological stress and growth (as positive or negative). In the current study, physical activity was the most important factor for both biological stress and growth. Physical activity played a more important role in growth (γ=0.698) than for biological stress (γ=0.546) according to this model, meaning that growth is affected by the level of physical activity more than biological stress in broiler chicken. Strong correlations were found between physical activity and biological stress and growth in some previous works (Torun & Viteri, 1994; Farr et al., 2013; Alves & Alves, 2019). As the number of birds per m^2 was relatively small in the current study (6 chicks), it was thought that there was little stress in chickens. A negative relationship between stocking density and biological stress was also found in previous studies (Simitzis et al., 2012). Also, the reason why physical activity affects stress less than growth could be due to the fact that chickens did not have any problem with movement. Accordingly, it can be said that this experimental study complies with EU welfare standards for broiler chickens (Augère-Granier, 2019). There was a poor and non-significant effect of biological stress on growth (γ=0.176), implying that possibly because of the low ratio of total oxidant status/total antioxidant status of this construct in the current study. Moreover, the level of ratio showed that the animals in the experiments could be healthy (Simsek et al., 2009). Growth was significantly affected by exercise, whereas growth was not influenced by biological stress. The model illustrated a considerable proportion of the variance in growth (R^2=0.65) and a smaller proportion of the variance in biological stress (0.298). Therefore, R^2 values were two times higher for broiler growth than for biological stress, indicating a more applicable qualification of growth for the assessment of performance, in view of this data set. Growth and weight values can be used as a criterion to decide the grade of welfare, that has obtained value particularly in intensive poultry production farming.

Conclusions

The implementation of the PLS-SEM technique for poultry welfare assessment is in its first steps of improvement in welfare modeling. Measurements of animal welfare depend on such as body composition, cognition, dietary nutrient levels, emotion, category of animal, physiological status, age, physical activity based on environmental conditions, immune system, genetics and genomics, management, health conditions, and biological stress. This technique made possible to estimate growth, including the different approaches as latent variables which are physical activity and biological stress. The method was also able to estimate biological stress from physical activity. Further research is needed to confirm whether the PLS-SEM method can provide an advantage in terms of welfare quality. In the PLS-SEM, it is difficult to assign reasonable measurements to the relevant latent variables in order to meet the suitability requirements and to make accurate estimates. Future research, in addition to the existing latent variables, different latent variables such as the immune system and welfare, which are meaningful measurements, are needed to establish more comprehensive and complex models.

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Author Contrubitions: Mehmet Çetin designed the study and set up the experiments, Mehmet Çetin and Mehmet Göçmen conducted the study, Abdurrahim Koçyiğit did the biochemistry analysis, Hamza Yalçin analyzed the data, Hamza Yalçin, Zeki Doğan and Sedat Şen wrote the article.

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