

EVALUATION OF THE PHYSIOLOGICAL AND
BEHAVIOURAL RELATIONSHIPS OF YEARLING
BUCKS BY GENERALIZED PROCRUSTES ANALYSIS

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Abstract

The aim of this study is to evaluate the relationships between some sexual behaviours and physiological traits such as serum testosterone concentration, body weight loss and presence of horns of yearling bucks using Generalized Procrustes Analysis (GPA). In the study, 11 yearling bucks being sexually inexperienced were used. The sexual behaviours were determined with a total of four tests, which consisted of exposing the yearling bucks individually to three unrestrained estrous ewes for 15 min. In these tests, some behavioural characteristics such as number of ejaculations, flehmen response, number of mounts and serum testosterone concentration were determined. In the GPA analysis, the first two factors explained approximately 98% of the total variation between animals (Dimension 1 = 95.57%, Dimension 2 = 2.68%). It was determined that there was a positive and high correlation between tests and the number of mounts, which had a high correlation with the first dimension. Ejaculation frequency, flehmen response and testosterone levels decreased in line with increases in the number of mounts. As a result, it can be suggested that GPA can be effectively evaluated quantitative and qualitative behavioural characteristics without requiring any prior assumptions and reducing variability among animals.

Key words: qualitative behaviour assessment, consensus, preference map, correlation

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Introduction. In bucks, the sexual behaviour is one of the most important factors affecting the profitability in commercial goat farming in terms of herd breeding and efficiency. The male fertility is an important issue as numerous does are generally mated with a single buck [1]. Therefore, it is necessary to reduce the negative effects of sexual behaviour in increasing pregnancy rates. Accordingly, it is a good practice to assess sexual behaviour and sperm quality using a serving capacity test prior to breeding to achieve reproductive success. Many factors such as physiological status and sexual experience can be effective on sexual behaviours depending on the genotype, age, herd management and practices [1-3]. The aforementioned physiological and behavioural factors affecting the sexual behaviours have a very complex structure. For this reason, sometimes different approaches are needed in subjects such as examining the relations between the factors, evaluating and classifying animals individually.

The animal behaviour studies impose certain limitations on the characteristics of individuals studied, study designs, and statistical analysis of data according to different disciplines. Limited sample size is one of the most obvious constraints encountered. This sample limitation raises some statistical problems about the precision, accuracy or stability of the statistical analyses applied.

While a small sample size can provide reliable information in basic statistics such as measures of central tendency (for example, mean or median), measures of variability (for example, variance and standard deviation), reliable results may not be obtained in methods such as determining differences between groups or relationships between variables [4]. In addition to the insufficient number of samples in behavioural science, the other problem is that many behavioural characteristics are obtained by counting or consist of a large number of zero values. Such observations that do not show any normal distribution are assumed to have a hypothetical Poisson distribution. The most important feature of this distribution is that its mean is equal to its variance. However, it is very difficult to achieve this equality in count data, because the variance of the count dependent variable tends to increase depending on the widening of the count range (excess variability or heterogeneity). Therefore, the variability increases and causes the problem of overdispersion ($\text{Var}(Y_i) > \mu$) [5,6]. More complex models are needed to evaluate such data (for example, Generalized linear mixed models). However, in addition to many advantages of these models, many rigid assumptions such as the homogeneity and independence of errors, the ability to add important independent variables to the model, the presence of collinearity among the estimation variables, and the adequacy of sample size need to be investigated [6].

In this study, we propose a generalized Procrustes analysis that does not require any rigid assumptions for behavioural studies and can be used in different data structures (e.g. count, binary or continuous) and in small sample situations. In particular, the GPA method is free of assumptions about data distribution found in other existing approaches [7]. The method is more often used in sensory

analyses (see for example [8,9]). However, in behavioural studies, the Free Choice Profiling (FCP) method of different observers is also used to develop a common methodology from the terms they describe the behaviour of their animals (see for example [10–12]).

Materials and methods. Goat behaviour data. The animals used in the study were sexually inexperienced 11 (SI; average age 1.5 years; first service season) yearling bucks (Saanen $\times$ Hair goat crossbred bucks) and 12 Hair goat ewes (average age 4.1 years). The study was carried out over a period of 30 days at the beginning of the breeding season. During this time, all yearling bucks were with ewes, except on the days when the sexual performance tests were done. Four sexual performance tests were performed as described by [13]. The visual and olfactory contacts between the bucks and ewes was prevented before the sexual performance tests. The tests were carried out in a 4 $\times$ 5 m paddock by penning yearling bucks individually with three unrestrained estrous ewes for 15 min. The bucks were tested every other day between 07:00 and 13:00. To eliminate the effect of the day, the yearling bucks to be tested were randomly selected. The following behaviours were determined for all yearling bucks: flehmen response (FR), number of mounts (NR: attempts to mounts or mounts without pelvic oscillation), latency to first mount (FM: the time from entry into the pen to the first mount), reaction time (RT: the time between the exposure of a male to estrous female and ejaculation), number of ejaculations (NE), and ejaculation efficiency (EE). Ejaculation efficiency was calculated as follows: Ejaculation efficiency = mounts with ejaculations/(mounts with ejaculations + mounts without ejaculations). Sexual behaviour was observed by two researchers; while one researcher observed the behavioural characteristics, the other recorded the data. The presence or absence of horns was recorded for each yearling buck. Body weights of animals were recorded at the beginning and end of the experiment. The testosterone concentrations (TC) of yearling bucks were determined using blood samples taken twice on the same day (the same day with body weight measurements) from the vena jugularis of each male goat. The 10 mL of blood was taken at 07:00 and 17:00 on each sampling day. Blood samples were centrifuged at 3000 $\times$ g for 15 min, then serum was collected and stored at -80°C until analysis. Serum testosterone concentrations were tested using a goat testosterone commercial ELISA kit (CSB-E13630G, Cusabio Biotech Co. Ltd., Wuhan, China) according to the manufacturer's instructions. The detection range of the kit is 0.1–20.0 ng/mL and the sensitivity is 0.05 ng/mL. The coefficient of variation of the kit is below 15%. Samples with a higher testosterone concentration than the highest standard were diluted with phosphate-buffered saline.

Procrustes analysis. In this part of the study, the individual data matrices of 11 animals were created. While there are four tests in the columns of each matrix, in the rows the case of being horned or not for animals (0 or 1), weight loss and as sexual behaviour characteristics; the number of ejaculations, flehmen

response, number of mounts, and testosterone concentration took place. Therefore, the individual matrices for each animal were formed from four columns and six rows. A consensus configuration matrix was obtained by mean 11 individual matrices.

Different variable structure in GPA offers the opportunity to evaluate together, because it compares distances between the features obtained from different measurement structures (for example; count, continuous variable, binary variable, etc.) in matrices formed from individuals [10]. Thus, it is not affected by the heterogeneity or extreme variation in behavioural data. Three different Procrustes transformations (translation, isotropic scaling and rotation/reflection) are used to obtain a common consensus configuration from individual matrices containing behavioural characteristics obtained from different measurement structures (qualitative or quantitative) of animals. The purpose of transformation is to combine the same features and transform them into a geometric configuration. Thus, it transforms the different individual matrices of 11 yearling bucks into a multidimensional consensus configuration. Consequently, the variability is reduced by matching between the features and a common consensus configuration is obtained [14,15]. All of the interpretations are made over this consensus configuration. In order to make interpretations easier, the dimensions of the consensus configuration are reduced through Principal Component Analysis (PCA). The PCA determines which are the main axes of consensus and how much of the variation each of these axes explains among animals. It then ensures that the major axes of the consensus configuration are given meaning (semantics). The meaning of the main axes is not made based on the behavioural characteristics. It is based on the relationship of the coordinates of the consensus configuration to the coordinates of each of the 11 yearling bucks' individual matrices. If the consensus profile of a behavioural characteristics is highly correlated with the first or second axis of the matrix, that axis is associated with that behavioural characteristics. Making sense of axes with behavioural characteristics facilitates interpretation [10,12]. XLSTAT software (Free Trial version) package programs were used for GPA analysis.

Results and discussion. In the first stage of the Procrustes analysis, the effect of all three transformations applied in order to combine the common behavioural characteristics of 11 yearling bucks and reduce variability was found to be significant scaling ($F = 29.31$; $p < 0.0001$), rotation ($F = 2.83$; $p < 0.0001$) and translation ($F = 2.90$, $p < 0.0001$). Therefore, by removing the individual effect of each animal, the properties were combined and the first step of the consensus configuration was taken. The next steps of the Procrustes analysis are based on the results of the consensus configuration obtained after these transformations [16]. Since the consensus configuration is based on the average of the individual matrices of 11 yearling bucks, the results for the error variations of the consensus configuration are presented in Table 1 for both the overall and individ-

T a b l e 1

Residuals for behavioural characteristics and goats

Characteristics	Residuals	Animals	Residuals
Flehmen response	276.280	Animal 1	92.507
Number of mounts	574.400	Animal 2	166.035
Number of ejaculations	196.220	Animal 3	100.880
Presence of horns	228.166	Animal 4	81.347
Testosterone concentration	427.056	Animal 5	204.891
Weight loss	289.216	Animal 6	92.884
		Animal 7	100.577
		Animal 8	48.913
		Animal 9	739.534
		Animal 10	88.820
		Animal 11	274.950

ual evaluation of the behavioural characteristics. Also, the individual errors that allow the examination of the compatibility of each animal with other animals in terms of sexual behavioural characteristics are given in Table 1.

When Table 1 is examined, it can be seen that ejaculation frequency error variation is the lowest characteristics of the sexual behaviour characteristics. Accordingly, in the four tests applied to animals, it was determined that all animals showed ejaculation efficiency in a small number, since the ejaculation efficiency had the lowest error. The sexual performance effectiveness in animals may vary depending on experience. In parallel with the results of our study, some studies show that older males have higher ejaculation efficiency than sexually inexperienced male and female goats [17,18]. However, testosterone concentration with higher error (0.083–7.408 ng/mL) and number of mounts (0–74) were the two characteristics that varied the most between the animals in the tests. According to [19], the error variance of each animal in Table 1 reveals the relationship between the individual sample map created from the sexual behaviour measurements of each individual and the consensus sample map (formed from the data obtained from 11 animals). The animals' error variance provides a measure of each animal's fit within the consensus domain. Accordingly, four out of 11 animals (Animals; 2, 5, 9 and 11) showed less agreement than the other animals since they had a high error variance indicating heterogeneity between the animals. It is possible to say that there is a high agreement among other animals with lower error values.

The first two dimensions explained approximately 98% of the variation between animals in sexual behavioural characteristics obtained from different measurement structures (Dimension 1 = 95.57% and Dimension 2 = 2.68%) (Fig. 1). However, in behavioural studies involving qualitative characteristics the disclosure rates of the first dimension vary by 24–70% [10–12]. Therefore, it is consid-

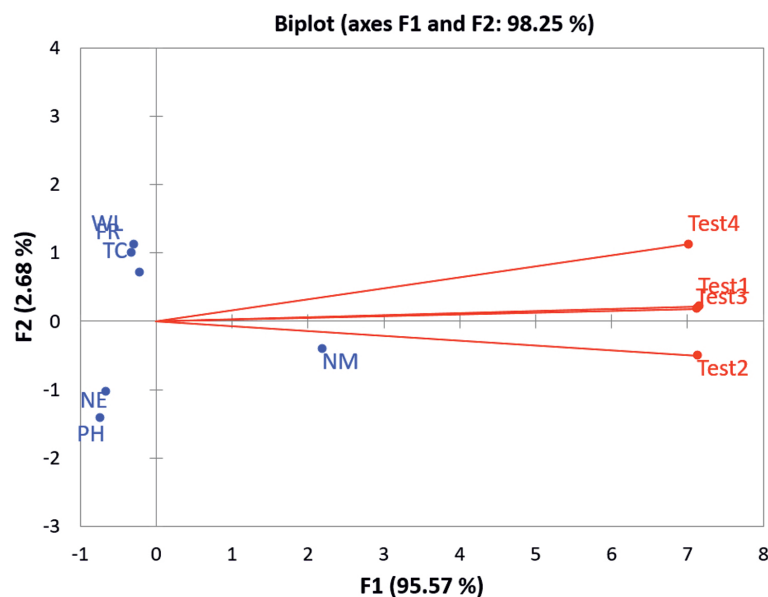


Fig. 1. Correlations between the tests and behavioural characteristics. F1: First principal component of GPA; F2: Second principal component of GPA; FR: Flehmen reaction; NM: Number of mounts; NE: Number of ejaculations; PH: Presence of horn; TC: Testosterone concentration; WL: Weight loss

ered that 98% of the explained variance is quite sufficient to present them in a two-dimensional space in our study, which includes features with both qualitative and quantitative measurements. According to Fig. 1, it was determined that the correlation between the tests was high for all four tests and there was a positive and high correlation between the tests and the number of mounts. Similarly, with increasing number of mounts, ejaculation frequency, flehmen response, weight loss and decrease in testosterone levels were determined.

Two different consensus configuration maps are given in Fig. 2. In the first, the relationship of individual behavioural characteristics with consensus configuration, and the second allowed us to examine the relationship of behavioural characteristics with consensus configuration in general. When consensus maps are examined, it is clear that there is agreement that the number of mounts are separated sharply on the map, and the animals are not able to ejaculate, so there is a consensus that they mount more. At the same time, it is seen that the first dimension has a high rate of explaining variation among animals, about 95.57%, and the number of mounts are located in this region on the map. The ratio of the dimensions explained, the number of mounts have a very high contribution. Other features are not clearly separated on the map.

The increase in the proportion of variance explained among animals makes it possible to obtain clearer distinctions between behavioural traits in the consensus

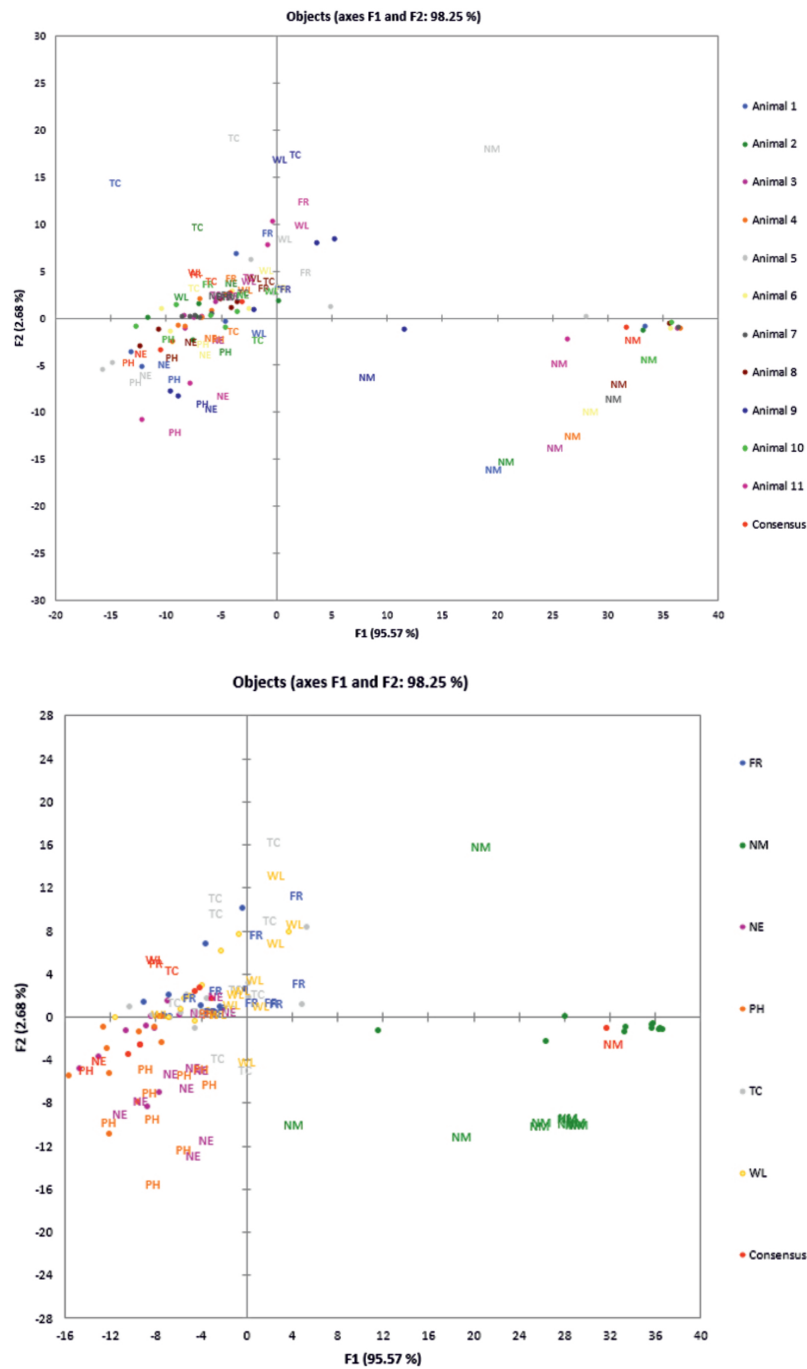


Fig. 2. Individual configuration map of animals and configuration map of behavioural characteristics. F1: First principal component of GPA; F2: Second principal component of GPA; FB: Flehmen response; NR: Number of mounts; NE: Number of ejaculations; PH: Presence of horn; TC: Testosterone concentration; WL: Weight loss

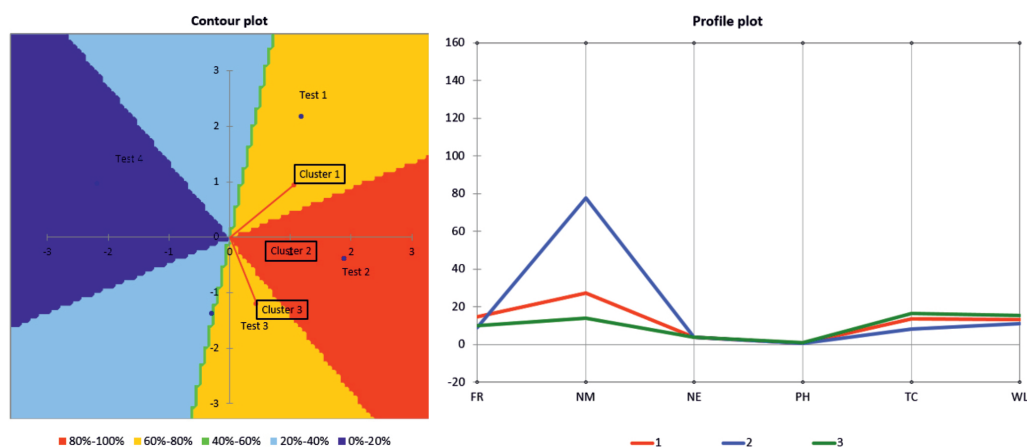


Fig. 3. Preference mapping illustrating three clusters: 1, 2 and 3 and profile plot of animal behaviour characteristics

map. Consensus map which explains 78% and 69% of the variation among animals in terms of various behavioural characteristics, could make a clear distinction between behavioural expressions. In another study examining free choice profiles in quantitative behavioural traits, it was reported that the two dimensions of the consensus map explained 22% of the total variation among animals, and the terms used for behaviours by observers clustered at the origin due to the low proportion of variance explained [10]. Therefore, a distinction could not be made on the map due to the observers' use of the same and similar terms.

In general, the preference maps are widely used to determine the preference rates of homogeneous consumers for each food sample in sensory analyses [20]. In our study, in order to evaluate the performance of animals divided into three homogeneous groups in terms of behavioural characteristics at test times, the preference map is given in Fig. 3, while the prominent behavioural characteristics of the animals in the clusters are presented.

When Fig. 3a, b are examined together, they show the flehmen response 60–80% more in the animals in group 1 than in the animals in the other two groups at the time of the 1st test. It is seen that the number of mounts is higher in animals in group 2 because 80–100% ejaculation could not be performed at the second test time. It can be said that the animal in group 3 have a slightly higher testosterone concentration and weight loss of 60–80% at the time of the third test compared to the animals in the other group. At the fourth test time, the animals did not exhibit any prominent behaviour compared to the other test times.

Conclusion. In this study, individual behavioural and physiological characteristics of animals were examined by GPA analysis. A very large proportion (approximately 98%) of the total variation among animals was explained by the first two dimensions. The contribution of the number of mounts is high in this explanation rate. Since most of the animals could not perform ejaculation activity,

it was determined that they mounted more at all test times. As a result of this study, the GPA method can be recommended as a practical approach that can be used as an alternative method to other statistical approaches in behavioural datasets.

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