AN APPLICATION OF HOTELLING'S T^2 TEST FOR THE COMPARISON OF THE VISUAL-ACOUSTIC METHOD IN THE IDENTIFICATION OF INGESTIVE CATTLE BEHAVIOR

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■ ABSTRACT: The bioacoustic method is an important tool for the identification of the ingestive behavior of ruminants, especially in extensive production systems. This is mainly due to its potential to solve the deficiencies presented by the usual method, which is based on the visual observation of the animals. In this article, we present a study whose main objective is to evaluate the accuracy of the bioacoustic method over the visual method to record the macroactivity of grazing cattle ingestive behavior. The comparison of the methods is made in terms of a multivariate statistical approach based on the use of Hotelling's T^2 test. To verify the test performance in comparing the methods, we developed a simulation study using a resampling approach. The results show that the bioacoustic method can be an effective alternative to the visual method, with the advantage of being a noninvasive method that also allows the analysis of the micro events of ingestive behavior.

■ KEYWORDS: Bioacoustic method; visual pasture; multivariate analysis; Holtelling's T^2 test.

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1 Introduction

In extensive beef production systems, there is a great interest in methods to identify and analyze the ingestive behavior of cattle. This interest is due to the fact that the ingestive behavior of cattle is a quantitative and qualitative indicator of its feed as well as of the physical environment that it is in. Due to this, an accurate methodology to determinate the ingestive behavior of animals is an important tool.

According to Setz (1991) the visual observation is the usual method to verify the ingestive behavior of the cattle. However, as discussed by Souto (2005), this method may present some limitation, such as:

  (i) the need for more than one evaluator, favoring errors of subjectivity;

 (ii) the need for constant attention of the evaluator, making the method exhaustive and, consequently, compromising the correct registration of the activities;

(iii) complete measurement made only during the use of a special device;

(iv) controversial information about the ideal time interval for registration, complicating the comparison between research results.

Due to these limitations, the visual observation method is contested in the academic realm. Thus, there is a great interest in methods that combine precision in data collection, equipment resistance, low cost, and non-invasiveness. Under this perspective, the bioacoustic method is an effective alternative due to its practical and economic potential, besides solving the limitations of the usual method (DECRUYENAERE *et al.*, 2009, LACA, 2009, EXADAKTYLOS *et al.*, 2014, ALVES *et al.*, 2017).

In this paper, we present a study whose main objective is to evaluate the accuracy of the bioacoustic method, in relation to the visual method, for the macro-activity record of ingestive behavior of cattle on pasture. For this, we develop an experimental study, in which, the average time that an animal is observed in the activities of grazing, ruminating or other activities[1] is simultaneously measured by the visual and bioacustic method. Our hypothesis is that the average time that the animals were observed in these activities by both methods does not have significant statistical differences.

In order to make a decision on the hypothesis, we consider a multivariate statistical approach and the Hotelling's T^2 test (HOTELLING, 1931). Besides, to verify the performance of the statistical test, we develop a simulation study using a resampling approach (EFRON, 1981; DIACONIS; EFRON, 1983). The results show that the bioacoustic method may be an effective alternative to the visual method with the advantages of allowing continuous recording of activities, eliminate the need for observers and consequently reducing errors and costs, among other benefits (CLAPHAM *et al.*, 2011).

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[1]idleness, ingestion of mineral salt, ingestion of water, and scratching on trees.

The remainder of the paper is structured as follows. In Section 2, we describe the time in study and how visual and acoustic methods are used in the experimental study. Section 3, presents the multivariate statistical approach, the Hotelling's T^2 test and the results for the real dataset obtained in the experimental study. Section 4 concludes the paper with final remarks. Additional details are provided in the Appendix when referred to in this paper.

2 Material and methods

The experimental study was carried out in April and May of the year 2016, during two consecutive days of each month, in the period from 8:00 am to 4:00 pm ($GMT + 4h$), at Embrapa Beef Cattle, located in the Campo Grande city, Mato Grosso do Sul, Brazil.

The study was conducted with twelve nelore (*Bos taurus indicus*) females with age between 28 and 32 months and weight between 350 and 410 kg. The procedure and methodology described in this paper were previously approved by the Embrapa Cattle Ethics and Animal Use Committee, under the registration number 013/2014. The animals were randomly distributed, in pairs, into six production system pickets crop-livestock-forest integration.

2.1 Visual method

The ingestive behavior was assessed by instant visual observation using the focal-animal sampling method (ALTMANN, 1974), with activity recording at 10-minute intervals (SANTANA *et al.*, 2012). To allow individual registration of the activities, in each picket, there was an animal marked with spray paint on both sides of the body, and another one unmarked. The evaluated activities were: grazing, rumination and other activities (leisure, mineral salt intake, water intake and scratching on trees).

The activities were recorded by two observers with experience in visual assessment of ingestive behavior, through the use of binoculars (Bushnell, 8×42mm). Due to the presence of trees in the environment, observers sometimes had to move to better visualize the animals.

2.2 Acoustic method

The evaluation of the ingestive behavior through the acoustic method was carried out simultaneously to the visual observation, following a methodology adapted from the literature; see for example Trindade *et al.*, (2012) and Laca (2009).

Each animal was equipped with a generic lapel microphone and a digital voice recorder (Sony, ICD-PX240) configured as follows: high-quality recording mode (HQ, MP3 128 kbps), "meeting" environment, low sensitivity microphone, low-cut filter activated and up to 24-hour alkaline batteries, according to the manufacturer's specifications and the configuration used. Regarding the allocation of the equipment, the audio recorder was placed in polyvinyl chloride (PVC, 75mm

x 15cm), closed with a windshield cover, in which, at one end, there was a hole for the microphone cable to pass through. The microphone was inserted into a Styrofoam capsule and positioned on the animal's forehead by means of a rubber band. The equipment was fixed to the halter with the aid of adhesive tape and nylon clamps and positioned at the nape of the neck, as shown in Figure 1.

Figure 1 - Bioacoustics equipment and their positions: (A) Recorder inside of the capsule of PVC; (B) Lapel microphone inserted in a Styrofoam capsule. (C) Microphone positioned in the front; (D) Capsule fixed to the vessel, positioned behind the marrafa.

As heifers were accustomed to the use of the halter, no adaptation period was required. However, in order to avoid any problem, the equipment was placed on the animals about 15 hours before the start of the experiment.

At the end of the experimental period, the equipment was removed and the sound records were transferred to the computer. Using Audacity® software, version 2.1.2, the time each animal was grazing, ruminating and other activities were quantified. No acoustic treatment was used to improve the sound record. Besides, there was also no prior knowledge of the data collected through the visual method.

Due to technical problems in the bioacoustic equipment installed in three animals, these animals were removed from the study. Thus, the statistical analysis was developed using the measurements obtained from nine animals.

3 Hotelling's T^2 hypothesis test

Let X_{ij}^v and X_{ij}^a be the average time that i-th animal was observed by the visual (v) and acoustic (a) method, respectively, in the activities: grazing (1), ruminating (2) or other activities (3), for $i = 1, \ldots, n$ and $j = 1, 2, 3$, in which, n is the number of animals in the study.

Consider $\mathbf{D}$ be a $n \times 3$ matrix with elements $D_{ij} = X_{ij}^a - X_{ij}^v$, for $i = 1, \ldots, n$ and $j = 1, 2, 3$. Denote by $\mathbf{D}_i = (D_{i1}, D_{i2}, D_{i3})$ the vector of observations from i-th line of the matrix $\mathbf{D}$, for $i = 1, \ldots, n$. Assume that

$$\mathbf{D}_i \sim \mathrm{N}_3(\boldsymbol{\mu}, \Sigma)$$

where $\mathrm{N}_3(\cdot)$ represents the three-variate normal distribution with mean vector $\boldsymbol{\mu} = (\mu_1, \mu_2, \mu_3)'$ and covariance matrix Σ, for $i = 1, \ldots, n$.

The interest here is to verify whether the average time measured by visual and acoustic methods present a statistically significant difference. This leads to the following hypothesis testing

$$H_0 : \ \boldsymbol{\mu} = \boldsymbol{\mu}_0 \quad against \quad H_1 : \ \boldsymbol{\mu} \neq \boldsymbol{\mu}_0,$$

for $\boldsymbol{\mu}_0 = (0, 0, 0)'$. The decision on the hypotheses must be made according to some statistical criterion. The following, we present the criterion based on Holtelling's T^2 test (HOTELLING, 1931).

Thus, let $\overline{\mathbf{D}} = (\overline{D}_1, \overline{D}_2, \overline{D}_3)'$ be the sample mean vector, in which, $\overline{D}_j = \frac{1}{n} \sum_{i=1}^{n} D_{ij}$ for $j = 1, 2, 3$, and $\mathbf{S}$ be the covariance matrix of the sample. The Hotelling's T^2 test statistic is given by

$$T^2 = n(\overline{\mathbf{D}} - \boldsymbol{\mu}_0)' \mathbf{S}^{-1} (\overline{\mathbf{D}} - \boldsymbol{\mu}_0). \tag{1}$$

This expression is obtained from the likelihood function of a multivariate normal distribution when H_0 is assumed true; see for example BILODEAU and BRENNER (1999), JOHNSON and DEAN (2007). The distribution of the T^2 test statistic under H_0 is related to the F-distribution according to the following expression,

$$F = \frac{n - p}{(n - 1)p} T^2 \sim F_{(p; n-p)}.$$

Thus, for a given significance level α, we reject H_0 if $F > F_{(1-\alpha,p,n-p)}$, where $F_{(1-\alpha,p,n-p)}$ is the quantile $1-\alpha$ of the F-distribution with p and $n-p$ degrees of freedom. In this case, we conclude that at least one of the three means is not equal to 0. In the remainder of the paper, we set up $\alpha = 0.05$.

In this study, it is also interesting to construct simultaneous confidence intervals for the parameter vector $\boldsymbol{\mu}$. According to Johnson and Dean (2007), an interval of $100(1-\alpha)\%$ of confidence for μ_j is given by

$$\overline{D}_j \pm \sqrt{\frac{p(n-1)S_{jj}}{n(n-p)}F_{(1-\alpha,p,n-p)}},$$

where S_{jj} is the j-th element of the diagonal of the covariance matrix $\mathbf{S}$.

3.1 Aplication

In this section, we apply the Hotelling's T^2 test to the real dataset obtained from the experiment described in Section 2. Table 1 shows the average time (in minutes) of the activities observed by each method.

Table 1 - Average time of activities obtained by both methods

Animal	Visual			Bioacoustic		
	Grazing	Ruminating	Others	Grazing	Ruminating	Others
1	43.7500	3.7500	12.5000	37.4000	5.6000	17.0000
2	45.0000	5.0000	10.0000	45.0000	2.7500	12.2500
3	46.2500	7.5000	6.2500	43.8000	6.2001	10.0000
4	40.0000	6.2500	13.7500	42.7500	7.0001	10.2500
5	36.2500	15.0000	8.7500	37.1250	13.2500	9.6250
6	33.7500	12.5000	13.7500	37.0000	9.8750	13.1250
7	46.2500	8.7500	5.0000	39.2000	11.4001	9.4000
8	42.5000	3.7500	11.2500	33.3333	0.0000	24.6667
9	47.5000	3.7500	6.2500	31.0000	0.0000	29.0000

The matrix of observed data is given by

$$\mathbf{D} = \begin{bmatrix} x_{11}^a - x_{11}^v & x_{12}^a - x_{12}^v & x_{13}^a - x_{13}^v \\ x_{21}^a - x_{12}^v & x_{22}^a - x_{22}^v & x_{23}^a - x_{23}^v \\ x_{31}^a - x_{13}^v & x_{32}^a - x_{32}^v & x_{33}^a - x_{33}^v \\ x_{41}^a - x_{14}^v & x_{42}^a - x_{42}^v & x_{43}^a - x_{43}^v \\ x_{51}^a - x_{15}^v & x_{52}^a - x_{52}^v & x_{53}^a - x_{53}^v \\ x_{61}^a - x_{16}^v & x_{62}^a - x_{62}^v & x_{63}^a - x_{63}^v \\ x_{71}^a - x_{17}^v & x_{72}^a - x_{72}^v & x_{73}^a - x_{73}^v \\ x_{81}^a - x_{18}^v & x_{82}^a - x_{82}^v & x_{83}^a - x_{83}^v \\ x_{91}^a - x_{19}^v & x_{92}^a - x_{92}^v & x_{83}^a - x_{93}^v \end{bmatrix} = \begin{bmatrix} -6.3500 & 1.8500 & 4.5000 \\ -0.0001 & -2.2501 & 2.2500 \\ -2.4500 & -1.3000 & 3.7500 \\ 2.7500 & 0.7500 & -3.5000 \\ 0.8750 & -1.7501 & 0.8750 \\ 3.2500 & -2.6251 & -0.6250 \\ -7.0500 & 2.6499 & 4.4000 \\ -9.1667 & -3.7499 & 13.4167 \\ -16.5000 & -3.7501 & -22.7500 \end{bmatrix}$$

At this point, before realizing the Holtellings' T^2 test, we need to verify the normality assumption for the observed data in matrix $\mathbf{D}$. For this, we apply the normality verification procedure described in Appendix 1. The normality verification shows that there is no reason to doubt of the normality of the data, despite the small sample size. Please, see Appendix 1 for more details.

Verified the normality assumption, we can proceed with the calculus of the test statistic. From matrix $\mathbf{D}$, the average vector $\overline{\mathbf{D}}$ and the covariance matrix $\mathbf{S}$ of the sample are given by,

$$\overline{\mathbf{D}} = \begin{bmatrix} -3.8491 \\ -1.1306 \\ 5.3130 \end{bmatrix} \text{ and } \mathbf{S} = \begin{bmatrix} 42.2321 & 2.7300 & -49.2481 \\ 2.7300 & 5.5418 & -9.2542 \\ -49.2481 & -9.2542 & 64.4579 \end{bmatrix}. \quad (2)$$

The average vector and the covariance matrix were obtained using the software R (R CORE TEAM, 2019) and the commands $apply(\mathbf{D}, 2, mean)$ and $cov(\mathbf{D})$.

The value of T^2 statistic is

$$T^2 = 9 \begin{pmatrix} -3.8491 & -1.1306 & 5.3130 \end{pmatrix} \begin{pmatrix} 7.4980 & 7.7244 & 6.8377 \\ 7.7244 & 8.1915 & 7.0783 \\ 6.8377 & 7.0783 & 6.2560 \end{pmatrix} \begin{pmatrix} -3.8491 \\ -1.1306 \\ 5.3130 \end{pmatrix}$$

$$= 6.1515;$$

and the F statistical value is $F = \frac{6.1515}{4} = 1.5378$.

Since the theoretical value $F_{0.95,3,6} = 6.5988$ is greater than the calculated statistic $F = 1.5378$, we do not reject the null hypothesis H_0. The corresponding p value is 0.2987. That is, we have no evidence to consider that visual and bioacoustic methods differ significantly in relation to the time measurements that animals are grazing, ruminating or doing other activities.

The confidence intervals (95%) for means μ_1, μ_2 e μ_3 are given by:

$$CI_{\mu_1}(95\%) \quad : \quad (-13.3066; 5.8307);$$
$$CI_{\mu_2}(95\%) \quad : \quad (-4.5536; 2.2924);$$
$$CI_{\mu_3}(95\%) \quad : \quad (-6.3609; 16.9867).$$

At this point, one can note that the three intervals contain the value 0. This indicates that there is no evidence for the difference between measurements obtained by both methods. This result shows that the bioacoustic method can be an effective alternative to the visual method; with similar precision and the advantage to be a noninvasive method.

3.1.1 Resampling approach

As the sample size is small, then we develop a resampling procedure to verify the suitability of the result. For this, we implement the following procedure. For the index $l = 1, \ldots, L$:

(i) Generate a random sample of size $n = 9$ from a three variate normal distribution with mean vector and covariance matrix given in (2). Here, we use the R software and the command *rmvnorm()*;

(ii) Calculate the test statistic T^2, get the statistical value F and the p-value;

(iii) Let $\mathbb{I}_l$ be an indicator variable, so that, $\mathbb{I}_l = 1$ if $p - value < \alpha$ and $\mathbb{I}_l = 0$ otherwise, for $l = 1, \ldots, L$;

(iv) Calculate the proportion of times $\hat{P} = \frac{1}{n_t} \sum_{i=1}^{n_t} \mathbb{I}_i$ that null hypothesis is rejected.

The resampling procedure was performed for $L = 10,000$. Figure 2 shows the boxplot the p-values for the L simulated cases. The minimum and the maximum p-values were 0.0001 and 0.9963, respectively; the median value was 0.1187 and the mean value was 0.1879. The first and third quantiles were 0.0379 and 0.2667, respectively.

The proportion of times that the null hypothesis H_0 was rejected is $0,292$. That is, in 70.8% of the simulated cases, the null hypothesis was not rejected; as in the real application.
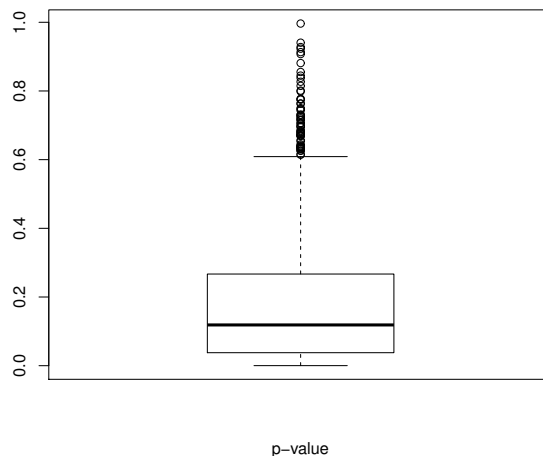

Figure 2 - Boxplot of the p-values.

4   Final remarks

This paper presents an experimental study to compare the visual method against the acoustic method, used to identify the ingestive behavior of cattle in meat production systems. For each animal its behavioral activity (grazing, rumination and other activities) was recorded via visual method at ten-minute intervals. Each animal was also equipped with an audio recorder and a microphone to obtain sound

recordings. To perform the statistical analyzes we considered the averages of the activity time of each animal in both methods.

In order to compare the measures from both methods, we adopt a multivariate statistical approach and a hypothesis test procedure. The decision procedure is based on the Hotelling's T^2 statistic test. A practical differential of this approach is that we compare in a joint way the average time measured in the three activities considered. In opposite to the usual approaches, which are based on the use of analysis of variances followed by the comparison of each activity separately using a univariate hypothesis test.

At the 5% significance level, the null hypothesis is not rejected. This indicates that there is no evidence for the difference between the mean time measured by the visual and acoustic methods. This result shows that the bioacoustic method can be an effective alternative to the visual method to identify the ingestive behavior activities of grazing cattle. In addition, the bioacoustic method has the advantage of being a noninvasive method that does not depend on an observer and allows the continuous recording of the activities. Therefore, eliminating the errors of subjectivity present in the visual method and thus contributing to the reduction of production costs.

The statistical analysis was implemented in Software R. The codes can be obtained by emailing the first author.

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■ *RESUMO: O método bioacústico é uma ferramenta importante para a identificação do comportamento ingestivo de ruminantes, principalmente em extensos sistemas de produção. Isso se deve principalmente ao seu potencial para solucionar as deficiências apresentadas pelo método usual, que se baseia na observação visual dos animais. Neste artigo, apresentamos um estudo cujo objetivo principal é avaliar a precisão do método bioacústico em relação ao visual para registrar a macroatividade do comportamento ingestivo de bovinos em pastejo. A comparação dos métodos é feita em termos de uma abordagem estatística multivariada, com base no uso do teste T^2 de Hotelling. Para verificar o desempenho do teste na comparação dos métodos, desenvolvemos um estudo de simulação usando uma abordagem de reamostragem. Os resultados mostram que o método bioacústico pode ser uma alternativa eficaz ao método visual, com a vantagem de ser um método não invasivo que também permite a análise dos micro eventos do comportamento ingestivo.*

■ *PALAVRAS-CHAVE: Método bioacústico; método visual; análise multivariada, teste de Holtelling T^2.*

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Appendix 1: Normality verification procedure

To verify the assumption of the normality of the data, we firstly use the graphical method q-q plot. This method consists of the plot the percentiles of the sample against the percentiles expected by the adjustment of a normal distribution. If the points can be described by a straight line, the assumption of normality does not be rejected. In the multivariate case, the construction of the q-q plot is based on the following result: If $\mathbf{D} \sim N_p(\boldsymbol{\mu}_p, \boldsymbol{\Sigma}_p)$, where $N_p(\cdot)$ represent a p-variate normal distribution, then $(\mathbf{D} - \boldsymbol{\mu}_p)^T \boldsymbol{\Sigma}_p^{-1} (\mathbf{D} - \boldsymbol{\mu}_p) \leq \chi_p^2(1-\alpha)$, in which, $\chi_p^2(1-\alpha)$ is the value of the percentile $(1-\alpha)$ of the chi-square distribution with p degrees of freedom. Thus, the construction of the q-q plot is given by the following steps:

(i) Calculate the sample square distance $dg_i^2 = (\mathbf{D}_k - \overline{\mathbf{D}})' \boldsymbol{S}_D^{-1}(\mathbf{D}_k - \overline{\mathbf{D}})$, for $i = 1, \ldots, n$;

(ii) Let $dg_{(1)}^2 < \ldots < dg_{(n)}^2$ be the quadratic distances values in an increasing numerical order;

(iii) Consider $F_{(i)} = (i - 1/2)/n$ be the empirical percentiles associated to the $dg_{(i)}^2$ and $q_{(i)} = \chi_p^2(F_i)$ be the theoretical percentile of the chi-square distribution with p degrees of freedom;

(iv) plot $\mathbf{dg}^2 = \left(dg_{(1)}^2 < \ldots < dg_{(n)}^2 \right)$ against $\mathbf{q} = \left(q_{(1)}^2 < \ldots < q_{(n)}^2 \right)$.

Applying the procedure described above for the real data set $\mathbf{D}$ presented on Section 3.1, we have that

$$\mathbf{dg}^2 = (2.1779, 0.7940, 0.3769, 3.1662, 0.6916, 2.0107, 3.3185, 4.4592, 7.0049).$$

Table 2 presents the ordered $\mathbf{dg}^2$ values and the corresponding $F_{(i)}$ and $q_{(i)}$ values, for $i = 1, \ldots, n$. Figure 3 shows the q-q plot.

Table 2 - Results of the $dg_{(k)}^2$, F_k e q_k

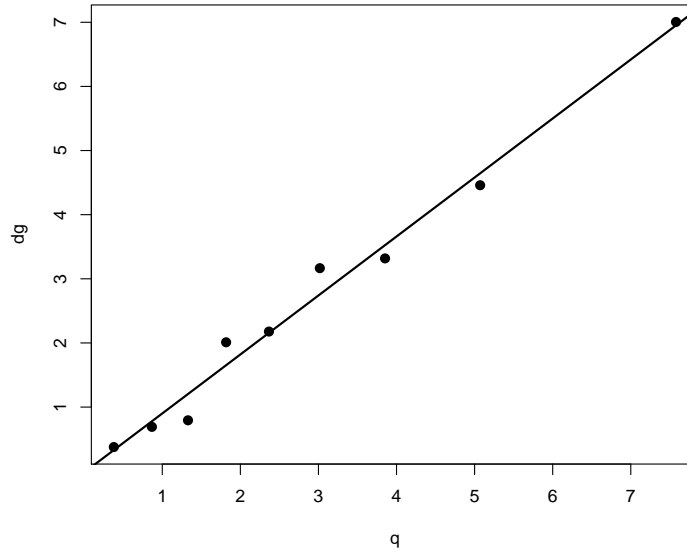| k	1	2	3	4	5	6	7	8	9
$dg_{(k)}^2$	0.3769	0.6916	0.7940	2.0107	2.1779	3.1662	3.3185	4.4592	7.0049
F_k	0.0556	0.1667	0.2778	0.3889	0.5000	0.6111	0.7222	0.8333	0.9444
q_k	0.3795	0.8672	1.3292	1.8176	2.3660	3.0178	3.8530	5.0711	7.5793

Figure 3 - Gráfico q-q plot.

As one can note in Figure 3, there is no reason to doubt of the normality of the data, despite the small sample size. In addition, we also calculate the linear correlation between the $\mathbf{dg}^2$ measures and the $\mathbf{q}$ quantiles. The obtained value is $r = 0.9923$, indicating a strong positive linear relationship. Thus, based on q-q plot and r value we do not reject the normality assumption for the observed data, despite the small sample size.

Besides the graphical method, we also apply the multivariate Shapiro-Wilk normality test. In this test, the null hypothesis affirms that the matrix of observed data is from a normal distribution. To realize the test, we use the package *mvnormtest* and the command *mshapiro.test(*$\mathbf{D}$*)*, where $\mathbf{D}$ is the matrix observed dataset described on Section 3.1. The p-value from the test is 0.2545. This result, show us that the normality assumption for the matrix of observed data is not rejected.