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STUDY OF THE SOCIALIZATION AND HUMAN INTERACTION ON THE IMPROVEMENT IN ANIMAL **BEHAVIOR IN DOGS HOUSED IN** THE INSULAR SHELTER OF GRAN CANARIA

VETERINARY DEGREE FINAL **DEGREE PROJECT**

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DEDICATION



I owe this work mainly to animals in general, which have been the driving force of my life since I was a little girl.

To the dogs in this essay, who live 24 hours a day behind a fence, and appreciate like nothing else the minimum affection that we offer them.

And mainly I dedicate it to Vanilla, I wish that her stay in heaven is infinitely more pleasant than the one she lived on earth, for you and for you my little Vanilla.



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ABSTRACT

Animal welfare is considered an important basis in veterinary medicine. It is essential to consider the effect that the human-dog interaction has on it, as well as the environment where the animals, in this case shelter dogs, live. Dog shelters tend to be characteristically stressful places for dogs, due to social isolation, unfamiliar environment, high noise, physical restriction, absence of routines or habits and limitations.

Objective: The aim of this study is to test whether or not there is an improvement in the welfare, behavior and quality of life in shelter animals experiencing environment enrichment and human interactions with positive reinforcement.

Materials and Methods: This study was carried out by using two groups of 5 animals specially selected for this work: A Group Treatment subjected to the human interaction and positive environment with ludic activities, and a B Group Control that will not experience variations in their routines, both groups were evaluated with a behavioral test and measurements of analytical and physiological parameters, these measurements were taken before and after the treatment.

Results: The evaluation of the animals showed significant variations between the measurements of both groups of (p-value = 0.01587) for the behavioral tests, (p-value =0.045) for the weight measurements, of (p-value <0.001) for the platelet measurements and finally of (p-value = 0.017) of the white blood cells.

Conclusions: The implementation of environmental enrichment measures, outside cage recreation and human interaction, it may be possible to improve at least some aspects of the quality of life and behavior of shelter dogs. However, we believe that it is necessary to go deeper into more specific studies and to evaluate our study variables independently, as well as to consider a larger sample of animals.



1 INTRODUCTION

1.1 The dog: origin and domestication

Domestic dogs (Canis lupus familiaris) are the oldest and most popular species of companion animals. The hypothesis of the origin of the dog concluding that their closest relatives are wolves, and that modern dog breeds were domesticated ten thousand years ago (Koler *et al.*, in 2015).

The domestication of the wolf occurred due to genetic and hormonal alterations and because the wolf found advantages for its survival, such as easy feeding, together with man (Romero C., 2007).

The dog evolved from the gray wolf, and he was the first domesticated animal and hundreds of breeds exist today. During domestication, dogs experienced strong selection for temperament, behavior, and cognitive ability and have been closely associated with humans for many centuries for the ability to build social relationships (Tonoike *et al.*, 2022)

Human beings have played a major role in creating dogs that fulfill distinct societal needs. Through the most rudimentary form of genetic engineering, dogs were bred to accentuate instincts that were evident from their earliest encounters with humans (Constance B., 2022)

Dogs are highly social animals and are well adapted to living in groups. Studies have also shown that they are very good at interpreting human gestures and behavior. Dogs interact with each other and people through body postures, facial expressions, tail, and ear positions, raising of hair or "hackles," vocalizations, and scents. Between 3 to 8 weeks of age, dogs tend to focus on other dogs (if available) for social interaction, and between 5 to 12 weeks of age they shift their focus to people. Dogs are most receptive to learning how to deal with new situations until about 16 to 20 weeks of age. After this age, dogs do not stop learning from exposure; they just do so at a much slower rate and perhaps in a different way (Landsberg, G.,2018).

People and animals have had close associations over time due to the human-animal bond. This bond is described as a mutually beneficial relationship between people and animals that is essential to the health and well-being of both (AVMA, 2018)



1.2 Animal welfare

In scientific terms, there is no universally accepted definition of animal welfare; however, those most commonly used encompass many of the same ideas and principles. There have been several definitions proposed in the scientific literature; for example, "the state of an individual (animal) with respect to its attempts to cope with its environment" (Broom, 1986).

There have been several definitions proposed in the scientific literature; for example, "the state of an individual (animal) with respect to its attempts to cope with its environment" (Broom, 1986).

Hughes (1976) defined it, from a positivist point of view, as the state of physical and mental health in which individuals are in harmony with their environment. Animal welfare is immersed in the realm of the subjective perceptions of the individual, and therefore cannot be determined directly, but it is possible to work on it and recognize it through scientific methods (Mateos, C., 1994).

In other domestic animals it is admitted its evaluation by means of the check of five great threats, denominated in English "Five Freedom" (Council, F., 1992), since they estimate to what extent the animal is:

- Free from hunger and thirst.
- Free from discomfort.
- Free from pain, harm, or suffering.
- Free from fear or stress.
- Free to express normal behavior.

It is now known that, in general, the trigger for most abnormal behaviors in different species is directly related to failures in adapting to the environment, whether physical or social, in which an animal has to live. The result of this maladaptation is what is known as negative emotional states, among which are anxiety, frustration in carrying out activities, fear, boredom and, in an almost terminal phase, a depressive state. Lack of routines, human social disorders with dogs, poor housing conditions and even lack of exercise, are all situations that can generate these negative emotional states. (Tamborrell, M., 2022).



1.3 Pet Shelters

Each year animal shelters receive, care for, and rehome dogs, cats, and other companion animals.

However, shelters can be challenging environments for the animals admitted. Often, animals are housed in unfamiliar locations with limited space and, typically, high noise levels (McGuire B., 2021).

Therefore, animal shelters can be stressful places for dogs to live. Social isolation is likely one component of the environment that contributes to poor welfare but spending time out of the kennel with a person has been shown to temporarily ameliorate that stress (Gunter, L., *et al.*, 2021).

The environment of canine shelters is characterized by the presence of a set of stressors, such as social separation, exposure to a new environment, as well as excessive noise (product of barking), physical restriction, alterations in light- dark and probably circadian. rhythm, disruption of daily habits, and general unpredictability and loss of control (Hennessy, Williams, Mellott & Douglas, 1997; Tuber *et al.*, 1999).

This is how shelters are, in many cases, factors that generate behavioral changes reported by their possible new families and that cause the animals to be returned to the institution. (Diesel, Pfeiffer & Brodbelt, 2008; Wells & Hepper, 2000a)

More recently, animal welfare organizations have been focusing on the standard of care that dogs receive while in the shelter (Chrousos, G.P, 2009). In part, this is in recognition of the potential stressors within the environment, including excessive noise (Bowman, A., *et al.*, 2015) spatial restriction, social isolation (Appelhans, B.; Luecken, L.J, 2006) loss of attachment figures, loss of control and lack of a daily routine (Pumprla, J, *et al*, 2002) . One way to mitigate the impact of these stressors is through the use of enrichment interventions intended to improve welfare. The most commonly studied enrichment intervention in sheltering is interactions with people. The majority of these interventions occur at the shelter but out of the kennel and are 15–45 min in duration. Often, their impacts are measured by changes in physiology and behavior (Rietmann, T., *et al.*, 2004).

The ability to assess these emotions in shelter dogs is extremely important, as they are directly related to animal welfare and adoption success (Tuber et al., 1999). It has also been reported



that some emotional traits displayed by dogs in the shelter may be predictive of welfare problems after adoption.

This review and study will serve to try to test the feasibility and possible effectiveness of the application of some enrichment strategies and therapies that can help improve the welfare and quality of life of shelter dogs.

2 JUSTIFICATION AND OBJECTIVES

Confinement in confined spaces such as cages usually generates a high degree of stress and behavioral pathologies in dogs, as well as a lack of socialization both with humans and with others of the same species.

Therefore, the objective of this trial was to compare whether or not there is an improvement in the welfare, behavior and quality of life of a treatment group of dogs from the Albergue Insular de Gran Canaria, who experienced outings to the park, environmental enrichment and interactions with a handler at least three times a week for two months, with respect to a control group of dogs that will not be taken to the park or stimulated in any way by a human.

3 MATERIALS AND METHODS

3.1 Location and length of the experimental study

The present study was carried out at the facilities of the Albergue Insular de Animales de Gran Canaria, located at Camino Rosa Silva, 61, 35415 Bañaderos, Las Palmas, Spain.

The experimental study had a duration of two months, performing human-animal interactions in the dogs of the treatment group, of approximately one hour per day for each animal, during 4 days per week. These dogs lived in cages with 2 meters of wide, 3.5 meters long and 3.0 meters high, with a distribution of a roofed space for shelter and an open space with access to the sun.



3.2 <u>Study subjects</u>

The individuals to study were dogs who lived in cages of the shelter; some live in groups of two or three animals per cage and others live individually.

For one hour a day, the modification was to get out of the cage to perform therapies, interaction with a human and recreation in an interactive park.

Initially, twelve dogs of different sexes, ages and breeds were selected to be divided in two groups (six animals per group). As shown in table 1.

However, later the groups were reduced due to two casualties, one of them died due to gastric torsion, and the other individual was adopted, so each group was reduced to five animals each. Five of them to be treat it and the other five as control group.

Dog Name	Group	Estimated age (Y)	Gender	Breed
Mariposa		3	F	Podenco (hound)
Lavanda	Treatment	3	F	Belgian Malinois
Sultán		7	М	Labrador Retriever
Massi	Treatment	9	М	Podenco (hound)
Duna		10	F	Podenco (hound)
Vainilla (†)		7	F	Podenco (hound)
Turrón		2	М	Podenco (hound)
Dalí		2	М	Podenco (hound)
Don	Control	3	М	Pitbull
Neska	Control	4	F	Pointer
Luna		5	F	Pointer
Bogo (adopted)		5	М	Podenco (hound)

Table 1. Groups of dogs.

Control Group

We only perform behavioral and socialization evaluation tests, weight evaluation, and a basic analytical measurement. These measurements were performed twice, the first time at the beginning of the experiment and the second at the end of the experiment.



Treatment Group

In this group we performed the same behavioral and socialization evaluation tests, weight measurement and basic analytical tests as in the control group, but we also applied environmental enrichment measures and direct interactions with humans, and measurements in decibels of the noise in the corridor of the cages of the animals.

3.3 <u>Behavioral and socializing assessment test (Davíd J Menor Campos, UCO 2012)</u>

The following tests consisted of a series of varied exercises, whose objective was to evaluate and measure the responses of the animals to the different activities and stimuli, scoring the responses from one to five, with the number one being the least desired or lowest score, and the number five being the best expected or highest.

The tests were performed to the dogs of both groups (control and treatment) and will be done in two different opportunities (initial and final), the first one before starting the therapies on 04/25/2022, and finally the last test at the end of the therapies on 07/07/2022.

For the behavioral and socialization tests, different materials were needed in addition to human interaction. Below, we present the instruments we used in our study:

- Dog collar and leash
- Brush
- Unknown room for the animals
- Toy ball
- Rope toy
- Dog food bowl
- Wet dog food and snacks
- Sound toy
- Umbrella
- Inflatable balloons

Behavioral test

Annex I present the behavioral test that we used, it has a total of twelve activities through which we will measure the responses of the animals in socialization and behavior, this test was performed in two initial and final opportunities as mentioned above.





3.4 Physiological and Analytical Evaluation Parameters

Two measurements of weight and analytical values were performed in both working groups. An initial measurement, prior to any enrichment and socialization therapy (in the case of the treatment group), and a final measurement after performing the corresponding therapies.

The parameters that we evaluated in the blood analysis were: Red blood cell count, Hematocrit, Hemoglobin, Platelets and White blood cell count.

3.5 Sound level meter

The tool we used for the measurement of this variable is a mobile application called Sonometer: db. meter, noise meter, together with a microphone adapted to the cell phone to capture a betterquality sound. Two measurements were taken each day of work with the animals in the treatment group, before and after going out to the park and using the environmental enrichment therapies.

The main objective of these measurements was to compare the noise level of the corridor where the cages of our treatment group are located, both before and after the exit to the park and the enrichment therapies, to measure in a certain way, through the intensity of the noise by the vocalizations, the level of stress of the animals.

3.6 Interactive park or playground

The shelter has an outdoor park, with a series of obstacle structures and a swimming pool, as well as toys for recreation, and lots of olfactory stimuli such as plants and soil over a wide area.

The dogs in the treatment group were moved from their cages to the interactive park, where they recreated four times a week, for about an hour a day each.

In the park, the dogs could explore the terrain freely, however, we used a long walking leash of approximately two meters, this leash was kept attached to the animal's collar, so that at the end of the walk we could hold them easily since some animals in our study were very distrustful and were very afraid, so this way we could easily hold them at the end of the recreation time to return to the cages.

In the same way, in this space we performed socialization exercises, such as brushing the coat, positive reinforcement with palatable treats or caresses after performing some exercise or order





such as entering the pool, climbing the ramps, or entering the tunnel, in order to reinforce the interaction and the bond between the human and the dog.

3.7 <u>Environmental Enrichment Therapies</u>

We have selected three support tools that we will see below, to work and promote the environmental enrichment and quality of life of the animals belonging to the treatment group of this trial.

Lavender essential oil

The protocol consisted in the application of four drops of Lavender essential oil on the edge of the lateral side of the ear, specifically at the hair. This application was made after the dog went out to the park, just when they returned to the cage, to try to favor the dog's state of relaxation.

Probiotic: Canine Fortiflora from Purina®

Supplying one sachet per day of Purina® Pro Plan Canine Fortiflora orally, approximately four times per week for twenty days, these sachets contain a powder whose function is to be live probiotics of an exclusive strain of a lactic acid bacteria of Enterococcus faecium SF68 (5x108 CFU/g). This product provides a healthy and balanced intestinal function, as well as, according to the manufacturer, promotes a strong immune system.

Comfortable and soft beds inside the cages

We introduced a padded bed in each cage of the dogs in the treatment group, as another environmental enrichment tool, with the purpose of increasing their comfort and providing warmth during rest times, to improve their daily stay at the shelter.



4 **RESULTS**

4.1 <u>Behavioral and socialization assessment test</u> (Davíd J Menor Campos, UCO 2012)

Next, we can observe the results obtained from the behavioral test, where the variable Group classifies the dogs into: Control Group (those that do not receive any treatment) and Case Group (those that receive treatment). We observe the values obtained in an initial test and a final test.

In the following table we find represented the results of both tests for all the animals in our study. The score for evaluating each test varies from 1 to 5, being 5 the maximum score and 1 the minimum, therefore, in the Global section we can observe the total score obtained for everyone in each test, being 60 the maximum number of points possible to obtain.

Perro	Grupo	Medida	MiCoP	MayCoP	PasC	HabDes	IntH	ManipC	JPelot	JTirAfl	DefC	JSonor	RspEstN	RspRd	Global
1	Ca	In	5	4	4	2	4	4	3	3	5	3	4	4	45
1	Ca	Fi	5	4	5	2	4	5	3	3	5	3	4	4	47
2	Ca	In	4	3	4	2	2	3	3	3	3	3	4	4	38
2	Ca	Fi	5	5	5	2	4	4	3	3	5	3	4	4	47
3	Ca	In	3	4	4	2	4	5	3	3	3	3	2	2	38
3	Ca	Fi	5	5	4	2	4	5	3	3	3	3	4	4	45
4	Ca	In	2	3	5	1	2	2	3	3	3	3	4	4	35
4	Ca	Fi	4	5	5	2	4	4	3	3	5	3	4	4	46
5	Ca	In	4	4	1	2	4	4	3	3	4	3	4	4	40
5	Ca	Fi	5	4	3	2	4	4	5	3	5	3	4	4	46
6	Co	In	4	3	1	2	2	3	3	3	3	3	2	2	31
6	Co	Fi	4	3	1	2	2	3	3	3	3	3	2	2	31
7	Co	In	4	3	3	1	2	3	3	3	3	3	2	2	32
7	Co	Fi	4	3	3	2	2	3	3	3	3	3	2	2	33
8	Co	In	4	4	4	2	5	5	5	3	4	3	4	4	47
8	Co	Fi	4	4	4	2	5	5	5	3	4	3	4	4	47
9	Co	In	5	4	1	2	4	5	5	3	5	5	5	5	49
9	Co	Fi	5	4	1	2	4	5	5	3	5	5	5	5	49
10	Co	In	5	5	5	2	2	3	3	3	4	3	5	4	44
10	Co	Fi	5	5	5	2	4	3	3	3	4	3	5	4	46

Table 2. Behavioral test results

Perro: number of each individual Grupo
Ca: Case Group
Co: Control Group Medida:
In: Initial Measurement
Fi: Final Measure
MiCoP: Fear and Trust with People
MayCoP: Handling and Collaring with People
PasC: Walking on leash HabDes: Unknown Room IntH: Interaction with humans ManipC: Handling and brushing JPelot: Ball Game JTirAfl: Tug of War DefC: Feeder Defense JSonor: Sound toy RspEstN: Response to Novel Stimulus RspRd: Response to noisy stimulus Global: Total Sum





The following figure shows the variation between the overall values of the initial and final tests for each individual per group. The blue lines correspond to the treatment group (subjected to different environmental enrichment therapies), and as we can see most of the individuals show an increase, which translates into a positive response or improvement in the final test with respect to the initial test of the behavioral test.

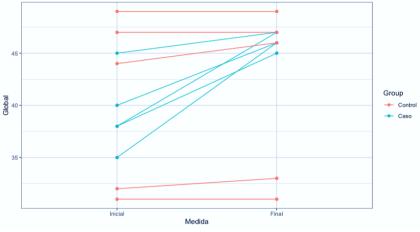


Chart 1. Global values of the behavioral test

However, the red lines corresponding to the control group, (which did not experience any variation in their routines or undergo any environmental enrichment therapy), remain constant or with a very slight variation on between the initial and final test.

The following *Chart* shows the evolution of the individuals with respect to each test performed in the behavioral test. Each colored line corresponds to an animal, on the "X" axis we have each of the tests and on the "Y" axis the variation of the initial and final score in each of them.

In the upper part, we observe the control group that barely shows variations or changes in the values, only individuals' number seven and ten reflected a modification in the behavior of their responses.

However, in the lower part of the *Chart* we can contrast the behavior of the results of the case or treatment group, which shows a greater number of modifications in the response of each variable evaluated for each individual.



R

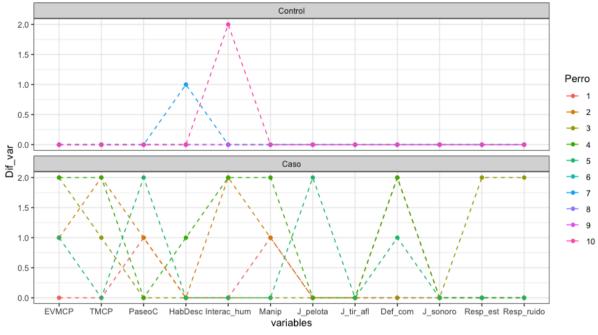


Chart 2. Individual response in the behavioral test.

In table 3 we find a summary of the results for each individual. In the change section it is possible to see the proportion of variation that each individual had, where it is possible to interpret a greater number of changes in the subjects belonging to the case or treatment group with respect to the control group.

Dog	Grupo	Mean_i (SD)	Mean_f (SD)	Dif_means	suma_i	suma_f	Cambio
1	Caso	3.75 (0.87)	3.92 (1.00)	0.17	45.00	47.00	2.00
2	Caso	3.17 (0.72)	3.92 (1.00)	0.75	38.00	47.00	9.00
3	Caso	3.17 (0.94)	3.75 (0.97)	0.58	38.00	45.00	7.00
4	Caso	2.92 (1.08)	3.83 (0.94)	0.92	35.00	46.00	11.00
5	Caso	3.33 (0.98)	3.83 (0.94)	0.50	40.00	46.00	6.00
6	Control	2.58 (0.79)	2.58 (0.79)	0.00	31.00	31.00	0.00
7	Control	2.67 (0.78)	2.75 (0.62)	0.08	32.00	33.00	1.00
8	Control	3.92 (0.90)	3.92 (0.90)	0.00	47.00	47.00	0.00
9	Control	4.08 (1.38)	4.08 (1.38)	0.00	49.00	49.00	0.00
10	Control	3.67 (1.15)	3.83 (1.03)	0.17	44.00	46.00	2.00

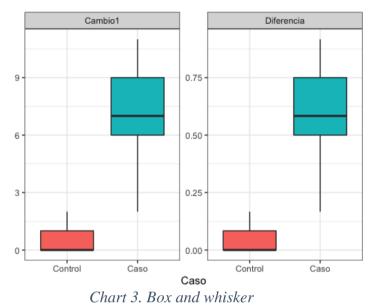
Table 3. Individual variations in test responses



- 1. **Dif_means:** The difference of means (final initial).
- 2. Sum_i, Sum_f: The sum of all initial and final measurements.
- **3.** Cambio: The difference obtained between the final and initial lump sum value (final initial).

Boxplots of the Change and Dif_means variables for the control group and the case group are shown below:

It is evident in both representations, that in both the change variable and the difference variable, the proportion of the case or treatment group is greater than that of the control group, which apparently means a greater improvement for this group (Case or treatment).



Wilcoxon Test

Finally, to check whether there are differences between the means of the values measured (globally) in each group, we used the Wilcoxon test as a statistical tool:

Whose result (p-value = 0.01587) indicates that between the mean value of the case group and the mean value of the control group there are significant differences.





4.2 Parameters of physiological and analytical evaluation

The results shown below correspond to the records of both weight in kilograms and the analytical variables studied for all the animals in our trial, expressed in two tables respectively.

In order to compare the evolution of the variables related to the analytical variables and the weight between the control and treatment groups and/or between times, **the data have been analyzed by means of a <u>mixed linear model</u> of the form:**

$$y_{ijk} = \mu + \alpha_i + \beta_j + (\alpha\beta)_{ij} + b_k + \varepsilon_{ijk}$$

Where:

 y_{ijk} is the value of the variable measured in dog k of group i at instant j, μ is the mean value of the variable at the start of the study, α_i s the group effect ($i \in \{\text{Control}, \text{Tratamiento}\}$), β_j is the effect of instant t ($t \in \{\text{Inicio}, \text{Final}\}$, ($\alpha\beta$)_{ij} is the interaction effect between group grupo *i* and time *j*, b_k is the individual effect of dog *k*.

The effects of group and observation instant are fixed effects, while the effect of the specimen is considered a random effect, so that b_k are independent and identically distributed random variables (v.a.i.i.d) with normal distribution $N(0, \sigma_b)$. In turn, the residuals ε_{ijk} are assumed to be also v.a.i.i.d with normal distribution $N(0, \sigma_{\varepsilon_i})$ (R Core Team , 2021).





Body weight

Regarding the weight of the animals that participated in the study, we were able to confirm that there were variations between the first and the last measurement, and also a difference between both study groups. In the following table it is possible to observe the weight obtained from all the animals:

CO= Control

TRA= Treatment

GROUP	ANIMALS	WEIGTH	
GROOT		INITIAL	FINAL
TRA	MARIPOSA	15,2	14,3
TRA	DUNA	24,3	23,4
TRA	SULTAN	26,5	25,6
TRA	MASSI	26,8	25,6
TRA	LAVANDA	23,7	23,3
СО	DALI	19,4	19,4
СО	TURRON	23,3	23,5
СО	DON	24,1	23,7
СО	LUNA	21	23
СО	NESKA	21,6	21,1

Table 4.Body weight results.

The results of the weight of the dogs were analyzed statistically and represented *Chart*ically, so that the *Chart*s allow visualizing the average evolution of each variable as a function of time, representing at the same time the data of both groups (control and treatment).

Significant differences between both groups Control and Treatment were obtained between the average weights measured at the beginning and at the end of the period of this trial. In particular, the following table is obtained from the statistical analysis carried out along the experience.



Fixed Effects Parameter Estimates

				95% Interval	Confidence			
Names	Effect	Estimate	SE	Lower	Upper	df	t	р
(Intercept)	(Intercept)	22.44	0.12	22.20	22.68	7.00	179.98	<.001
Group	TRA - CO	-0.54	0.26	-1.04	-0.04	7.00	-2.11	0.073
Tiempo	2 - 1	-0.30	0.24	-0.76	0.16	8.00	-1.27	0.239
Group * Tiempo	TRA - CO * 2 - 1	-1.12	0.47	-2.04	-0.20	8.00	-2.38	0.045

Table 5 Statistical weight analysis

The table 5 shows that the interaction between group and time (Group*Time) offers a **significant result (p-value =0.045).** This fact indicates that, given a constant time, the difference between the average weights in both groups presents significant differences (not attributable to chance).

*Chart*ically, these differences are also observed:

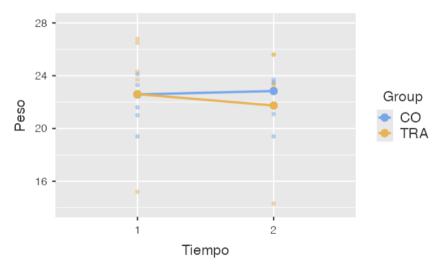


Chart 5 Weight averages for each group



White and red cell series

Once the blood samples were taken from the ten dogs in our trial, the first measurement on 02-05-2022 before starting to work with the animals in the treatment group, and a final sampling on 08-07-2022, we obtained the following results for the variables Red Blood Cell Count (RBC), Hemoglobin (HGB), Hematocrit (HTC), Platelets (PLT) and White Blood Cell Count (WBC).

GROUP	ANIMAL	RBC (M/uL) 5, 5-8,5				HCT (%) 37,3- 61,7		PLT (K/uL) 200- 600		WBC (K/uL) 6.0-17.0	
		in	fi	in	fi	in	fi	in	fi	in	fi
TRA	MARIPOSA	8,29	7,34	19	16,7	52,3	44,1	167	19	8,61	6,43
TRA	DUNA	9,28	9,09	20	19,1	56,8	55,1	237	26	8,98	12,68
TRA	SULTAN	8,08	7,86	18,4	18	52,7	50,6	142	36	13,39	12,54
TRA	MASSI	8,74	8,47	19,3	18,4	54,6	52,3	216	116	9,86	8,49
TRA	LAVANDA	8,24	8,86	17,9	21,2	49,9	54,8	189	20	17,19	9,94
СО	DALI	8,68	8,9	19,1	20,3	53,6	57,2	263	59	18,98	12,25
СО	TURRON	6,96	8,46	15,2	18,6	44	50,6	145	83	14,31	12,27
СО	DON	8,28	8,27	20,3	20,1	58,1	58,4	116	36	16,93	13,04
СО	LUNA	8,18	7,8	18,4	18,5	51,2	52,8	476	204	15,49	15,27
СО	NESKA	8,21	6,98	19,6	17	53,9	47	304	206	13,68	10,05

Table 6. Analytical Results.

CO: Control TRA: Treatment RBC: Red blood cell count HBN: Hemoglobin

HCT: Hematocrito PLT: Platelets WBC: Whithe blood cell count

The results related to the data obtained in the analytical tests: RBC, HGB, HCT, PLT and WBC were analyzed statistically and represented *Chart* cally, so that the *Chart*s allow visualization of the average evolution of each variable as a function of time, representing at the same time the data of both groups (control and treatment). It is important to note that the results obtained were not significant for the RBC, HGB and HCT variables, but were significant for the PLT and WBC variables.



The following table is obtained for the **<u>PLT</u>** variable:

					95% Confidence Interval			
Names	Effect	Estimate	SE	Lower	Upper	df	t	р
(Intercept)	(Intercept)	153.00	9.70	133.98	172.02	15.00	15.77	<.001
GROUP	TRA - CO	-21.06	20.73	-61.69	19.58	15.00	-1.02	0.326
Tiempo	2 - 1	-145.00	19.41	-183.04	-106.96	15.00	-7.47	<.001
GROUP * Tiempo	TRA - CO * 2 - 1	-3.60	38.82	-79.68	72.48	15.00	-0.09	0.927

Fixed Effects Parameter Estimates

Table 7 Statistical analysis of platelet measurements

From which it can be deduced that, for the fixed effects, there are significant differences for the variable Time. This result indicates that the mean PLT the values measured at beginning and the mean PLT values measured at the end present significant differences (p-value <0.001). These differences are shown in the following Chart:

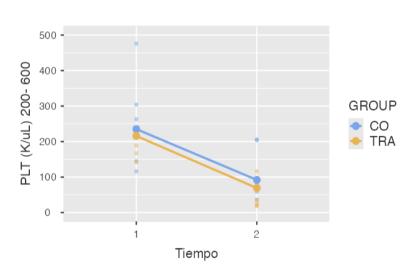


Chart 6 Platelet averages for each group





100% of the dogs in our trial presented a decrease in platelet values in the second sampling compared to the first sampling, individually we can observe these results in the following

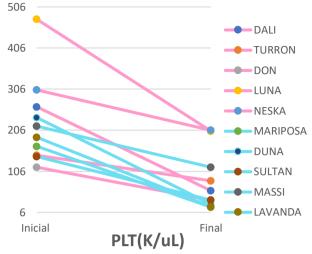


Chart 4. Result of platelet values for each dog.

The following table is obtained for the **<u>WBC</u>** variable:

				95% Interval	Confidence			
Names	Effect	Estimate	SE	Lower	Upper	df	t	р
(Intercept)	(Intercept)	12.52	0.45	11.63	13.41	15.00	27.56	<.001
GROUP	TRA - CO	-0.83	1.16	-3.11	1.45	15.00	-0.72	0.485
Tiempo	2 - 1	-2.45	0.91	-4.23	-0.67	15.00	-2.69	0.017
GROUP * Tiempo	TRA - CO * 2 - 1	1.71	1.82	-1.85	5.27	15.00	0.94	0.361

Fixed Effects Parameter Estimates

Table 8. Statistical analysis of white blood cells.

We observed that, for the fixed effects, there are significant differences for the variable Time. This result indicates that the mean WBC values measured at the beginning and the mean WBC values measured at the end present **significant differences** (**p-value** = 0.017).



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The following *Chart* shows these differences:

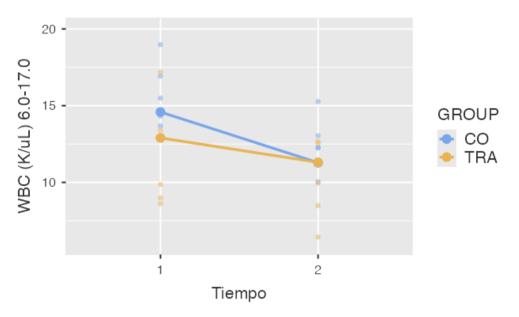


Chart 5. White blood cell averages for each group

Similarly, we can see that for the control group, 100% of its individuals presented a decrease in WBC values in the second sampling with respect to the first one, and the treatment group presented an 80% decrease in their WBC values, these individual changes are represented below:

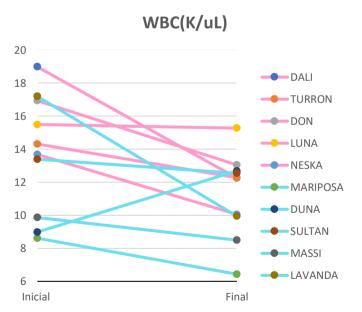


Chart 6 Result of the values of white blood cells of each dog.



4.3 <u>Sound level meter</u>

The following table shows the decibel measurements obtained during the experimental period. We can observe a first measurement that is taken in the corridor of the cages of the dogs of the treatment group before leaving the cage to the walk in the park and receiving their environmental enrichment therapies, and the second measurement corresponds to the moment after the aforementioned exit.

Once all the measurements have been taken, and a total average calculated for each measurement (1st and 2nd measurements), we can observe that for the first measurement the number is higher (50.40909091 dB) compared to the second measurement (40.33181818 dB).

Through the following *Chart* we can observe the differences of both lines of measurements corresponding to each moment of the day, the blue line corresponding to the first measurement before leaving the park, and the orange line represents the second measurement after leaving the park and returning to their cages each working day. Although we were not able to obtain statistical results for these variables, we can see a marked

	1st measure	2nd
Day	(dB)	measure
		(dB)
1	46	32,2
2	48,7	39,1
3	48,6	45
4	51,5	44,6
5	47,7	40,7
6	50,7	45
7	70	52,4
8	53,8	48
9	52,5	46,8
10	55	40,5
11	49,4	43,2
12	46,2	35,5
13	53,6	38,2
14	51,1	30,6
15	41,3	35
16	49,1	36,7
17	46,3	39,4
18	48,4	38,1
19	53,6	42,4
20	46,7	40,2
21	50,1	35,6
22	48,7	38,1
	50,40909091	40,33181818

trend in the first measurements with respect to the *Table 9. Results of measurements with* second. *the sound level meter in decibels.*





In addition, it is important to highlight the fact that individually the measurements of the 1st sample are always higher with respect to the second measurements each day as it is possible to observe in the following *Chart*.

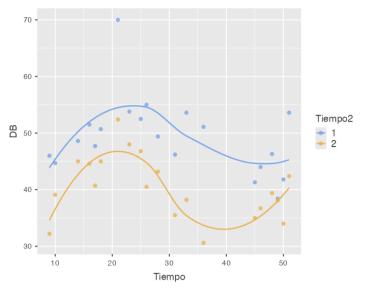


Chart 7. Results of measurements with the sound level meter in decibels

4.4 Interactive park or playground

The animals belonging to our treatment group were kept going out for one hour a day during each working day, approximately four days a week. Initially, some of them stayed away from the people in the park, and only maintained an interest in exploring the terrain and running.

However, over the course of a few days, the five animals belonging to this group (treatment) became more confident, eventually became more approachable with people, more accepting of handling, and more interested in interacting with people in the open recreational space.

4.5 <u>Environmental Enrichment Therapies</u>

Lavender essential oil

Each day of work, four drops of Lavender essential oil were applied on the edge of the lateral side of the ear at the level of the hair, in each animal belonging to the treatment group during the two months of testing. Initially the animals presented responses of fear and rejection to such manipulation and application, however after a few repetitions in time, their behavior became more docile and with great acceptance to such procedure, resulting visually calmer after the application.





Probiotic: Canine Fortiflora from Purina®.

With the administration of one envelope of Purina® Canine Fortiflora to each animal in the treatment group during the two months of the trial, four days a week. The most evident result obtained with this administration was the regulation of the defecation of the animals. Prior to the consumption of the probiotic, the feces were regularly soft. On the contrary, the animals of the control group kept their feces soft as usual in the Shelter due to the constant feeding changes.

Comfortable and soft beds inside the cages

With the introduction of a comfortable bed inside their plastic beds in each cage of the animals in the treatment group, we inspected the integrity of the beds every working day before going out to the park. However, after replacing the beds four times because they were biting, tearing and disintegrating them completely, we discarded continuing with the application of this tool, in view of the risks that are added with the possibility of oral ingestion of the tissues, as well as observing that they did not give the correct or expected use.



5 DISCUSSION

5.1 Behavioral and socializing assessment test

Behavioral tests are widely used for the general purpose of learning more about the animal, although they have their limitations (Menor, D., 2012). Thus, different authors argue that they only manage to expose a certain behavior, conditioned by the circumstances, which may change according to motivation, need, or previous experiences (Ley and Bennett, 2007; Mornement et al., 2010).

Despite the great influence that environmental conditions in shelters can have on the welfare of the animals residing there (Hennessy et al., 1997; Sales et al., 1997; Tuber et al., 1999), thus, can have a significant impact on their behavior (Hiby et al., 2006; Hubrecht et al., 1992; Beerda et al., 1999a), we have observed that there are many positive tools to benefit and improve the quality of life of dogs during their stay in a shelter. The advantages of this method are the possibility of studying the animal's responses to environmental challenges and the control of the situation (Manteca and Deag, 1993).

We felt great satisfaction in relation to the behavioral test, obtaining significant differences in the evolutionary responses between the initial and final test of both groups, but even so these are not immune to stress, social restriction and scarce human contact, so showing adequate, or at least acceptable, behaviors should be considered significant (Menor, D., 2012).

Usually, studies on canine behavior describe the tests to which the animals are subjected, however, the information is limited on the method of collecting the behavior, the behavior itself, or the assessment made of it (Jones, 2008). This idea is shared by different authors such as Diederich and Giffroy (2006); and Mornement et al. These authors carried out an investigation on the use of behavioral tests in dog shelters in Australia, gathering information on the procedures used, duration times, as well as interviewing those responsible for these processes. Unfortunately, they found a notable lack of objectivity in the measurements, a serious lack of standardization, and of course, an absolute lack of scientific validation of the instruments used.

Our results obtained in the behavioral test are in agreement with some studies of other authors, related to the interaction with humans through daily sessions of short duration (Hennessy et al., 2006, Hubrecht, 1993; Valsecchi et al., 2007) or single sessions of longer duration (Coppola, Grandin & Enns, 2006; Hennessy et al., 1997; Hennessy, Williams, Miller, Douglas & Voith,



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1998), in which a person talks to the dog gently, scratches its body and ears, caresses it and plays with it. Valsecchi et al. (2007) found that after 60 days of interaction with a human (through play, petting and obedience training), dogs showed better results in a temperament test, as well as in sociability and obedience evaluations, as opposed to control subjects. Therefore, it can be affirmed that these sessions decrease stress and improve the welfare of the dogs housed in the shelter (Menor, D., 2012).

5.2 <u>Physiological and Analytical Evaluation Parameters</u>

Body weight (Kg)

There is limited scientific evidence on studies of the relationship between weight of shelter dogs with exercise and human interaction. In our trial we obtained significant.differences in weight between both groups (control and treatment), where all animals in our treatment group presented a decrease in weight. Dogs kept in shelters are predisposed to weight gain because they are confined in restricted areas that limit physical activity, are usually subjected to gonadectomy and fed an unbalanced diet (Ricci R, et. al., 2007), in our case, dogs have food in their cages permanently, so their consumption is free throughout the day and they can consume more than they need, so we can observe some animals with a tendency to overweight.

Weight gain occurs when an animal accumulates a positive energy balance over a prolonged period of time. This can occur because energy intake increases and/or energy expenditure decreases; in our study, the remarkable number of obese and overweight dogs can be attributed mainly to the former (Ricci R, et. al., 2007).

Thus, we can consider that the decrease in weight in all animals in the control group is probably due to the increase in physical activity provided.

However, there are multiple factors in situ in the shelter, both environmental and external and internal, or even the animal's own health, which can undoubtedly affect variables such as weight in the animals. Some of these factors can be temperature changes, stress generated by the isolation and noise of the shelter, systemic diseases or changes in feeding. In spite of obtaining significant results in the differences of weights obtained from both groups in both





measurements, we consider that it is advisable to study these variables in more depth, with a larger sample number.

White and red cell series

As with weight, there is not much scientific evidence that directly relates the impact or influence that socialization and exercise therapies in shelter dogs may have on analytical values or results. In spite of having obtained in our analyzed samples, significant differences between both groups for the variable platelets and white blood cell count, it has not been possible to make a functional comparison with other authors.

For their part, Dudley ES, et al., 2015, in their study on the effects of repeated petting sessions on leukocyte count and plasma cortisol concentration in dogs housed in a shelter, obtained large increasing immune responses, and elevated but decreasing plasma cortisol concentrations in shelter dogs. Daily 30-min petting sessions only affected cortisol values, so the clinical significance of petting for immune and health outcomes remains unclear.

On the other hand, despite obtaining significant results in platelet variations in both working groups, these values showed an exaggerated decrease in all the animals in the study.

However, according to other works such as Gilor S. and Gilor C. (2011), studied that blood collection techniques and improper sample handling can have a significant effect on the results of blood analysis. Small (even microscopic) blood clots and platelet aggregations can lead to false results, such as anemia, thrombocytopenia, and inaccurate coagulation test results. Contamination of specimens with tissue factor can initiate clotting and shorten clotting times.clotting times.

As a result of their work concluded that, One of the most important effects on theand manual platelet count and platelet count estimates is the presence of platelet aggregates/clusters. Therefore, platelet clumps often cause a false decrease in platelet count (pseudothrombocytopenia), which can have important consequences for patient care.



5.3 Sound level meter

The hearing of animals differs from that of humans; dogs (Canis familiaris) have much better hearing and can hear sounds up to four times quieter than can the human ear. Recent research shows that noise in dog kennels may be a welfare concern for the animals (Sales et al., 1997), but currently no policies regulate noise levels in dog kennels. Sound levels in animal shelters regularly exceed 100 dB. Noise is a physical stressor on animals that can lead to behavioral, physiological, and anatomical responses.

There are currently no policies regulating noise levels in dog kennels (Grandin T, et al 2006). It has long been documented that audible sound has profound psychological effects on nonhuman animals and disturbs the healthy equilibrium of the body (Wei, 1969).

With our results of the decibel measurements in the corridor of the cages of our animals of the treatment group, we obtained average levels between 40 and 50 decibels approximately, with the highest levels prior to the exit of the cages, and the lowest values after them. However, we can consider the fact that the levels obtained in our measurements are not precisely the highest that are usually obtained in this type of enclosures, which are around 90 - 100 dB.

To put this into context, 95 dB(A) is comparable to a subway train, 110 dB(A) is a jackhammer, and 120 dB(A) is a propeller aircraft; any sound in the 90 to 120 dB(A) range is considered to be in the critical zone and can be felt as well as heard (Key, 2000).

In the shelter environment, cortisol levels have been documented to be above normal, in some cases three times that of household pets (Hennessey, Davis, Williams, Mellott, & Douglas, 1997). We also found that in this instance (Coppola, Grandin, & Enns, 2006).

Clearly elevated cortisol levels are not solely due to noise levels, however they are a contributing factor; likewise, with our noise measurements in our trial, we have not been able to fully discern the origin of such sounds, as, despite finding our microphone located in the corridor of our study animals, the proximity to other animals is considerable and clearly can intervene, making it a complex and difficult to clean measurement.



5.4 Interactive park or playground

The results obtained with our work at the level of adaptation to new routines and implementing exercise together with human interaction in our dogs in the treatment group were positive. The greatest evidence of change presented by the animals was a decrease in fear with people, which translated to an increase in their confidence. Previous research suggests that dogs receiving exercise, play, and human interaction have decreased stress and improved some behaviors in a kennel environment (Bergamasco et al., 2010; Coppola et al., 2006; Normando et al., 2009; Shiverdecker et al., 2013). However, the research of Hetts et al. (1992).

An alternative view rejects additional exercise as an effective treatment for overactivity in kennel dogs and suggests that calm interactions between volunteers and shelter dogs may result in calmer dogs (e.g., ASPCA, "Enrichment in the Shelter," 2015c; Sternber, n / A). However, our work included both alternatives together, therefore, once the animals explored and recreated in the park, on their own initiative always sought direct interaction with the caregiver, with positive reinforcement, petting, brushing, and administration of snacks, the animals' trust was gained in not too long a time frame.

In another study in which 8 Beagle dogs were observed for four years confined in pairs in 2.4 m^2 cages and allowed to exercise outdoors, they showed urinary cortisol and creatinine levels representative of chronic stress. Placement of dogs in pairs rather than individually seems to have little effect on the level of chronic stress; however, dogs housed individually with a space of 2.1 m², an outdoor section of 5.6 m² and with possibilities to walk outside regularly for 90 minutes do not show values as high as those mentioned above, which could mean that exercise would improve the conditions of an animal that remains caged continuously. When increasing the size of a small cage (0.5-3.0 m²) and increasing it a little (1 - 1.6 m²), little effect on the exercise behavior of the dog was observed. Furthermore, no differences were found in the behavior of dogs housed individually in 4.13 m² or 6.83 m² cages (Hubrecht et al., 1992).

This suggests that either the dimensions of the cages do not influence much the active behavior in dogs, or that the increase in the size of the cage is not large enough to modify the behavior, the cages by themselves do NOT modify this activity. In fact, in a social animal, such as the dog, many other aspects of the environment can influence its behavior (Cancino, M.A, 2021).



5.5 <u>Environmental Enrichment Therapies</u>

Lavender Essential Oil

Our results in relation to the application of lavender essential oil as environmental enrichment, were obtained subjectively, with the observation of behavioral changes after the application of the oil. Thus, the frequency of behaviors associated with stress was reduced, and increased behaviors associated with the welfare of the animal (Wells, 2004). In the same way, (Taylor, K.D., et al. 2007), obtained that the dogs spent significantly more time sitting and lying down with a p < 0.05 during the treatment with lavender with respect to the control group.

(Graham et al. 2005), used essential oils diffused in a rescue shelter and found that dogs spent more time lying down and less time moving when exposed to lavender and chamomile oils compared to rosemary and peppermint oil and a control (no odor).

(Wells D, 2006) studied the effects of lavender on travel-induced arousal in dogs. Dogs were exposed to a lavender-impregnated cloth and a control (unscented) cloth while traveling by car. Dogs exposed to lavender spent more time resting and less time vocalizing and moving.

Another study in which dogs received a dermal application of lavender or a placebo for four 3.5 h periods while monitoring Heart Rate Variability, those exposed to lavender, there was a significant increase in HF potency and a significant decrease in heart rate, but only during the third and fourth periods, respectively (Komiya, M, 2009). These results suggest that topical exposure to lavender oil had some effect on vagal activity (Amaya V, 2020).

Therefore, information regarding the effects of environmental enrichment in kennels through olfactory stimuli may prove to be useful, thus broadening our understanding of how we can optimally house and manage these animals (Graham, 2005).

Probiotic: Canine Fortiflora from Purina®.

The shelter suffers the reality of presenting regular changes in the brands of feed administered to the animals, which is why food-borne diarrhea is very common and recurrent in most of the dogs residing there, thus, it was particularly easier for us to observe the great differences in the conformation of the feces of the animals of our two working groups, In this way, it was particularly easy for us to observe the great differences in the stool conformation of the animals of our two working groups, where those belonging to the treatment group presented a more



solid and formed stool conformation since they started ingesting the Purina® probiotic Fortiflora, in comparison with the animals of the control group that undoubtedly had frequent episodes of diarrhea.

This reality of the shelter on the frequent change of feeding is not an isolated fact, it is a reality that affects a large part of these organizations. Bybee S, et al., (2011), in conducting a study on the administration of Enterococcus faecium SF68 and the effect on diarrhea in shelter dogs, highlight that shelter staff anecdotally believe that the rate of diarrhea in dogs in the study period was lower than in previous years, as a consistent ration has been given in this study. In previous years, food donations from multiple food sources were used from multiple food sources, so the dog wards rarely received the same diet consistently and some donated diets could be of poor quality.

A larger study in dogs will be needed to further evaluate the effects of SF68 on diarrhea rates in shelters or other stressful environments (Bybee SN, et al., 2011)

There have been other studies on the administration of SF68 to dogs or cats housed in research facilities, such as that of Benyacoub J, et al., (2003), suggesting that this probiotic has potential immune-enhancing effects. As in their study, puppies fed SF68 were compared to a placebo group and were shown to have higher total plasma IgA concentrations (P 0.05), numerically higher fecal IgA concentrations (P 5 0.056) and higher concentrations of canine distemper virus specific antibodies. Plasma IgG and IgA

Also, there was evidence that SF68 probiotic enhanced B-lymphocyte production, because puppies fed the probiotic diet had a greater proportion of circulating mature B cells than did control dogs.3 One drawback to the study, Dr. Lappin noted, was that the investigators be-gan recording data after 10 weeks of probiotic use. Therefore, the question remained whether significant immune enhancement could occur in even less time. (Benycoub J, *et al.*, 2003).

In response, Lappin M, et al. (2009), designed a study to examine the effect of *E faecium* SF68 supplementation on immune function in apparently healthy young adult beagles. In this experiment, the dogs were evaluated before and after 4, 8, and 12 weeks of supplementation, with the post-supplementation results compared with baseline values. The data demonstrated evidence of immune modulation of both T and B lymphocytes within 4 weeks of probiotic use.

The immune system plays an important role in preventing the adherence of inappropriate bacteria to enterocytes, with secretory IgA playing a particularly significant role (Suzuki K, et





al., 2010). In a previous study with dogs supplemented with the probiotic Enterococcus faecium (SF68®), faecal IgA levels were shown to increase (Benyacoub J,et al.2003) our results indicate that a highly digestible gastrointestinal diet in combination with a proven probiotic such as FortiFlora® (SF68®) could be considered for nutritional management of this disorder (Rallis, T. S., et al., 2022).

Comfortable and soft beds inside the cages

Chewing can be observed in many different contexts and can serve different functions. Destructive chewing is considered a nonspecific clinical sign that is either a normal behavior, e.g., oral exploration behavior, or related to a pathological condition, e.g., separation anxiety or compulsive behavior (Horwitz, 2018).

In our study, several contexts potentially leading to frustration/anxiety/high arousal, such as cancellation of activities (e.g., walks), change in daily routines, exciting events (e.g., veterinarian visit, dog training, or presence of visitors) were moderately related to object chewing. This relationship occurred in all age groups and further indicates that dogs may use chewing to cope with negative emotions (Arhant,C, 2021).



6 CONCLUSIONS

- With the implementation of environmental enrichment strategies, outdoor recreation and human interaction, it may be possible to improve at least some aspects of the quality of life and behavior of shelter dogs.
- However, it is important to highlight that although we observed significant differences between both groups, in Body weight gained, Blood White Cell and Platelets our results were no conclusive and further studies have to be carried out in order to obtain clear results improving the size of the sample. Therefore, we can consider our study as a pilot trial which guides us to undertake an experience including a larger sample.
- Welfare of shelter population is an important matter that it has to be considered for the current coexistence of its members. This experience shows the need to focus on behavior problems and based on the results obtained from this first experience it is very encouraging to keep applying this socialization therapies in the future. Nevertheless, more specific studies are needed to evaluate our study variables independently and with a larger sample of animals.



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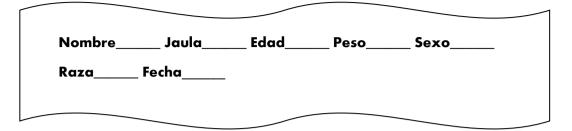




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8 ANNEXES



1.- Evaluación del Miedo y Confianza con personas: aproximarse a la jaula y esperar fuera

- 1. () Gruñe; Enseña los dientes; Se eriza; Castañetea; Se abalanza; Muerde; Ladra agresivamente
- **2.** () Se queda en el fondo de la jaula. Postura agachada, intenta escapar, se refugia en cubil, tiembla, pero no agresivo
- 3. () Se queda en medio de la jaula o se acerca a los barrotes Se queda quieto observando
- **4.** () Se aproxima nervioso
- 5. () Mira a la persona, se aproxima, mueve el rabo y saluda

2.- Tolerancia a la Manipulación y Confianza con personas: Poner el collar en la jaula

- 1. () Gruñe Enseña los dientes; Se eriza Castañetea Muerde Ladra agresivamente
- 2. ()
- 3. () Se arrincona, pero deja que se lo ponga; Se eriza
- 4. () Muy inquieto, salta, saluda; Se abalanza
- 5. () Se deja poner el collar sin protestar

3.- Paseo con correa:

- 1. () se revuelve contra la correa y no podemos guiarlo
- 2. () no se mueve; ladra constantemente
- **3.** () Camina dando brincos Salta sobre el guía Se revuelve contra la correa, pero podemos guiarlo
- 4. () Tira de la correa Se va cruzando No responde a la orden Junto
- **5.** () va bien

4.- Habitación desconocida:

- **1.** () no se mueve, o explora muy poco
- 2. () explora toda la habitación, muy entretenido

5.- Interacción con humanos, disponibilidad de crear vínculo con humanos: La persona le indica varias órdenes básicas sucesivamente, premiando amistosamente la respuesta

- 1. () Gruñe Enseña los dientes Se eriza Castañetea Se abalanza Muerde Ladra agresivamente
- 2. () Postura agachada, intenta alejarse físicamente, tiembla, pero no agresivo.
- **3.** () No sabe nada
- 4. () Viene a la llamada Nos sigue-junto y cuando cambiamos de dirección
- 5. () Sabe sentarse Sabe tumbarse Sabe quedarse quieto





6.- Manipulación: cepillado

- 1. () No se deja Gruñe; Enseña los dientes; Se eriza; Castañetea; Se abalanza; Muerde
- **2.** () No se deja, inquieto e impaciente; No se deja, agachado temblando, intentando escapar, NO agresivo
- **3.** () Se deja, inquieto e impaciente; Se deja, inmóvil, agachado; temblando, intentando escapar, NO agresivo
- 4. () Se deja inmóvil y tranquilo
- 5. () Se deja muy contento

7.- Juego con pelota:

- **1.** () si intentamos coger la pelota del suelo, gruñe, se eriza, muerde
- 2. () si intentamos quitársela de la boca gruñe, se eriza, muerde
- 3. () Va a por ella, pero no la trae La ignora, no sabe jugar o no juega
- **4.** () La trae, pero no suelta o suelta difícil No suelta, gira la cabeza, pero no se va No suelta, gira la cabeza y huye
- **5.** () Da la pelota si se la pedimos Podemos quitársela de la boca suelta fácil Podemos cogerla del suelo cuando está cerca

8.- Juego de tira y afloja:

- 1. () gruñe agresivamente, enseña los dientes, se eriza, muerde
- 2. ()
- 3. () no sabe jugar
- 4. () gruñe pero mueve el rabo, no está erizado
- 5. () juega sin gruñir

9.- Defensa del comedero

- 1. () Enseña los dientes Se eriza Castañetea Se abalanza Muerde Ladra agresivamente
- 2. () Gruñe
- **3.** () no le interesa
- 4. () deja que se lo quitemos, pero muestra ansiedad, come rápido.
- 5. () se lo deja quitar tranquilo

10.- Juego con juguete sonoro

- 1. () Gruñe Enseña los dientes Se eriza Castañetea Se abalanza Muerde Ladra agresivamente
- 2. () Gruñe
- **3.** () No sabe jugar
- 4. ()
- **5.** () se lo deja quitar tranquilo; le motiva el juego

11.- Valoración de respuesta a un estímulo novedoso (Paraguas):

- 1. () Gruñe Enseña los dientes Se eriza Castañetea Se abalanza Muerde Ladra agresivamente
- 2. () Se asusta, se agacha, tiembla, intenta huir
- 3. ()
- 4. () Se asusta, pero no huye
- 5. () Se muestra indiferente





12.- Valoración de respuesta a un ruido fuerte:

- 1. () Gruñe Enseña los dientes Se eriza Castañetea Se abalanza Muerde Ladra agresivamente
- 2. () Se asusta, se agacha, tiembla, intenta huir
- **3.** ()
- 4. () Se asusta, pero no huye5. () se muestra indiferente

