

ASSESSMENT OF STRESS IN RABBITS AND SOCIO-EMOTIONAL BEHAVIOR IN CHILDREN WITH AUTISM IN RABBIT-ASSISTED THERAPY

EVALUAREA STRESULUI LA IEPURI ȘI A COMPORTAMENTELOR SOCIO-EMOȚIONALE LA COPIII CU AUTISM ÎN TERAPIA ASISTATĂ DE IEPURI

Ana Maria ZAGRAI (MAIEREAN)^{1),4)},
Iuliana CODREANU²⁾, D.C. COTOR¹⁾, G. ZAGRAI^{1),3)},
Eniko CUPȘA⁴⁾, G. COTOR^{2),*)}, A. DAMIAN¹⁾¹⁾

ABSTRACT | REZUMAT

The impact of stress on the health of both humans and animals, as well as the main strategies to reduce it, is a topic of great interest to researchers in this field. The use of animals in therapies that include their assistance is becoming more and more widespread. Unfortunately, most research in this field focuses on humans, ignoring the assessment of the physical and mental health of the animals involved. Social deficiencies are a major challenge for people with autism. Integrating an animal as an additional method for children with ASD is based on the concept that an animal can act as a social mediator, thus helping to improve their social skills. The main aim of the study was to objectively determine the possible degree of impact of stress on rabbits caused by therapy-specific activities in children with autism spectrum disorders. The second objective of the study was to evaluate the impact of an existing TAA program on social communication skills, executive functions, and motor skills in children diagnosed with ASD. In our experiment, we used 16 clinically healthy rabbits (10 females and 6 castrated males) of the Netherland Dwarf breed. The 15 rabbits were aged between 5-7 years. The rabbits used in the experiment formed a uniform group, both in terms of maintenance and health. The study also involved 16 children (10 boys and 4 girls) who were recruited from the School Centre for Inclusive Education "LACRIMA" Bistrița. The ages of the children involved in the study ranged from 7 to 13 years old, diagnosed with ASD. The data obtained were statistically analysed, calculating the Mean and Standard Deviation (SD) using the Microsoft Excel application. At the same time, the statistical significance of the differences between the batches was calculated using the t-test (Student). The results highlight the fact that the use of rabbits as auxiliaries in the therapy of autism spectrum disorders in children determined a transient polycythaemia, which diminished by the end of the experiment, supporting a possible eustress to which the animals were subjected. The effect of TAI on hyperactivity indicate a decrease of 31.5%, of inappropriate speech a decrease of 21.42%, of stereotypes by 66.6%, of compliance by 57.80% and an improvement of attention in activity by 30.30%. And the biggest difference was found in the case of the Communication, where the difference was 93.75%. We argue that complementary therapy of children with autism spectrum disorder with the help of rabbits is an effective form of intervention, which can bring substantial improvements in therapeutic outcomes.

Keywords: rabbit-assisted therapy, stress, children with autism, haematocrit, complementary therapy

Impactul stresului asupra sănătății atât a oamenilor, cât și a animalelor, precum și strategiile principale de diminuare a acestuia, constituie un subiect de mare interes pentru cercetătorii din acest domeniu. Folosirea animalelor în terapiile care includ asistența acestora devine tot mai răspândită. Din păcate, cele mai multe cercetări din acest domeniu pun un accent pe om, ignorând evaluarea stării de sănătate fizică și mentală a animalelor implicate. Deficiențele sociale reprezintă o provocare majoră pentru persoanele cu autism. Integrarea unui animal ca metodă suplimentară pentru copiii cu TSA se bazează pe conceptul că un animal poate acționa ca un mediator social, ajutând astfel la îmbunătățirea abilităților lor sociale. Scopul principal al studiului a fost de a determina în mod obiectiv gradul posibil de impact al stresului asupra iepurilor, cauzat de activitățile specifice terapiei în cazul copiilor cu tulburări din spectrul autist. Al doilea obiectiv al studiului a fost de a evalua impactul unui program TAA existent asupra abilităților de comunicare socială, funcțiilor executive și abilităților motorii la copiii diagnosticați cu TSA. În experimentul nostru am utilizat 16 iepuri (10 femele și 6 masculi castrați), clinic sănătoși, din rasa Netherland Dwarf. Cei 15 iepurii au avut vârste cuprinse între 5-7 ani. Iepurii utilizați în experiment au format un grup uniform, atât din punct de vedere al stării de întreținere cât și al sănătății. La studiu au mai participat 16 copii (10 băieți și 4 fete) care au fost recrutați de la Centrul Școlar pentru Educație Incluzivă (C.S.E.I.) "LACRIMA" Bistrița. Vârstele copiilor implicați în studiu au variat între 7 și 13 ani, diagnosticați cu TSA. Datele obținute au fost analizate statistic, calculându-se Media și Deviația Standard (SD), folosind aplicația Microsoft Excel. Totodată s-a calculat semnificația statistică a diferențelor dintre loturi cu ajutorul testului t (Student), folosind aplicația Microsoft Excel. Rezultatele experimentului nostru scot în evidență faptul că folosirea iepurilor ca auxiliari în terapia tulburărilor din spectrul autist, la copii, a determinat o policitemie tranzitorie, care s-a diminuat până la finele experimentului, susținând un posibil eustres la care au fost supuse animalele, numai în prima parte a experimentului. Din cercetările noastre, rezultă că asocierea TAI la terapia curentă a copiilor cu TSA a lăsat copiii o amprentă semnificativă asupra parametrilor luați în evidență în studiul nostru. Observațiile noastre privind efectul TAI, asupra hiperactivității, indică o diminuare cu 31,5%, a vorbirii inadecvate o scădere cu 21,42%, a stereotipurilor cu 66,6%, a complianței cu 57,80% și o îmbunătățire a atenției în activitate cu 30,30%. Iar cea mai mare diferență s-a constatat în cazul Comunicării unde diferența a fost de 93,75%. Ceea ce ne face susținem faptul că terapia complementară a copiilor cu tulburări din spectrul autist cu ajutorul iepurilor, constituie o manieră eficientă de intervenție, care poate aduce îmbunătățiri substanțiale ale rezultatelor terapeutice.

Cuvinte cheie: terapie asistată de iepuri, stres, copii cu autism, hematocrit, terapie complementară

- 1) University of Agricultural Sciences and Veterinary Medicine, Faculty of Veterinary Medicine, Cluj-Napoca, Romania
 - 2) University of Agronomical Sciences and Veterinary Medicine, Faculty of Veterinary Medicine, Bucharest, Romania
 - 3) Saint Sylvester Secondary School, Bucharest, Romania
 - 4) School Center for Inclusive Education (C.S.E.I.) "Lacrima", Bistrița, Bistrița Năsăud county, Romania
- *) Corresponding author: cotor@yahoo.com

The impact of stress on the health of both humans and animals, as well as the main strategies to reduce it, is a topic of great interest to researchers in this field. It is a well-known fact that stress experienced during the development or transport period can significantly influence the health of animals. Also, stress is considered a factor associated with various conditions that occur naturally in humans. (31, 42).

Animal-assisted therapy for children with autism (AAT) is a form of complementary therapy that integrates techniques from various other therapies. TAA refers to a set of interventions that are based on the behavioural traits of animals, the most commonly used being dogs. These interventions can be integrated, as needed, into common treatments such as behavioural therapy, play therapy, and others.

The most common animals used in therapy globally are dogs, due to the fact that they are easy to tame and evaluate from a behavioural point of view, not needing specially designed locations or other special conditions. Due to their traits, these animals encourage effective communication with humans, manifesting themselves through a predisposition for play and the initiation of social interactions, as well as similarities in body positions that may reflect certain emotions or states of anxiety.

Animal-assisted therapy programs typically involve a wide range of animals, including dogs, cats, horses, rabbits, and others. Depending on the patient's needs, the scope can be: motor, cognitive, emotional, social, etc. (21). Each animal brings its own unique set of qualities and benefits to therapy sessions. Understanding the different types of animals used in therapy is essential for tailoring interventions to the specific needs and preferences of children with autism.

Other species of animals are also used, depending on the patient's needs (functional field of address: motor, cognitive, emotional, social) and the possibility of maintaining the animals: dolphins, farm animals (e.g., dwarf pigs, llamas) (21). The stress response is a normal part of daily life and is only harmful when triggered too intensely or for too long (49, 15).

The general adaptation syndrome, produced after brief exposure to low or medium intensity stressors, has a positive effect, inducing stress and improving the body's adaptive capacities (5, 8, 27, 34). Exposing animals to strong stressors or over a long period of time has the effect of inducing distress. It has negative implications that can lead to a pathological evolution (12, 33, 35). According to the American Veterinary Association's definition, the bond between humans and animals is a "mutually beneficial relationship that is essential to the well-being of both." However, animal welfare is of lesser importance compared to human welfare (20). Fear occurs when there is a sense of danger and causes changes in behaviour (11). In cases of sustained fear, chronic stress develops that deteriorates animal welfare (36).

It is known that the general adaptation syndrome, produced after short exposure to low or medium-intensity stressors, generally has a positive effect, indu-

cing stress, and improving the body's adaptive capacities (16, 18, 32).

Children with ASD need guidance towards their behavioural problems that can be improved or even prevented by providing attention, structure, clarity, predictability, safety, space, and peace (6, 7, 39, 43, 47, 48). At the same time, the well-being of children with ASD depends on their social and physical environment (50), they need more understanding, more time to respond (23) and less sensory input (51). In addition, each child has individual needs, depending on his or her cognitive abilities and the possibilities of making contact with reality (19). Children with ASD also create serious problems for their families (6, 47) because these families report high levels of stress (37, 41, 44). Therefore, to reduce stress levels, their families need to find ways to improve social-emotional behaviours (13, 14, 38), which in some cases could even help prevent family breakdown (1, 38).

The main aim of the study was to objectively determine the possible degree of impact of stress on rabbits caused by therapy-specific activities in children with autism spectrum disorders. The second objective of the study was to evaluate the impact of an existing TAA program on social communication skills, executive functions, and motor skills in children diagnosed with ASD. Unlike previous studies that primarily explored changes over time, this study was designed to explore what level of interaction with rabbits, if any, was associated with observed behavioural differences and other abilities during that interaction.

MATERIALS AND METHODS

The experimental protocol imagined by us lasted 10 weeks. During this period, with a frequency of 2 sessions per week, the TAI was initiated.

The duration of one session was 2 hours, with a break of 10 min. (10.00-12.00). For this purpose, 16 rabbits and 10 children diagnosed with autism spectrum disorders (ASD) were used.

The team that worked on this project was a multidisciplinary one, made up of volunteers – humans and rabbits – which included therapist specialists (psychologists, psychopedagogues, psychotherapists, speech therapists), animal-assisted therapy teams – veterinarians, but also other specialists who work to show people the benefits of human-animal interaction but also to popularise animal-assisted therapy and activities.

The experiment was carried out with the agreement of the School Centre for Inclusive Education © S.E.I.) "LACRIMA" Bistrița. The families of these children were notified about the conduct of this experiment within the C.S.E.I. and expressed their agreement, in writing, for the children's participation in this approach. The experimental procedures imagined did not affect the level of animal welfare.

Animals and experimental design

In our experiment, we used 16 rabbits of the Netherland Dwarf breed, with body weights of 1.5 ± 0.2

kg. The 16 rabbits were aged between 3-5 years (10 females and 6 castrated males). The rabbits used in the experiment formed a uniform group, both in terms of maintenance and health. The rabbits were separated into 2 experimental batches: batch 1 (Control; n=8) and batch 2 (Experimental; n=8), according to Table 1. During the experimental period the rabbits were housed in cages (95 × 57 × 46 cm) adapted to the species. The cages were kept in closed spaces. The animals endured a cyclical variation of the ambient temperature placed between 18-22°C. The relative humidity ranged between 40 and 50%. Food and water were administered at discretion. The feed consisted of compacted feed prepared according to a special recipe for rabbits, which contained 18% protein, 14% fibre, 2% fat and 2600 kcal/kg feed. The water was administered at discretion. The rabbits were kept in a lighting regime of about 10 hours/24 hours.

Before the animal-assisted therapy sessions, all rabbits (faeces) were checked by the veterinarian for the possible presence of parasites (e.g., *E. cuniculi*, *Passalurus* sp.). A physical examination was also performed, including a dental condition check and an assessment of the appearance of *Spilopsyllus cuniculi* and *Psoroptes*. The rabbits' claws were cut to avoid injury. All rabbits were free of zoonosis and parasites, and all animals were vaccinated against myxomatosis and haemorrhagic disease of rabbits (RHD).

Table 1
Table of animals used in the experiment

Animal nr.	Lot	Sex	Age (years)
1	1	Female	3
2	1	Female	5
3	1	Male	5
4	1	Female	4
5	1	Male	4
6	1	Female	5
7	1	Male	3
8	1	Female	5
9	2	Male	5
10	2	Female	5
11	2	Male	4
12	2	Female	4
13	2	Male	3
14	2	Female	3
15	2	Female	5
16	2	Female	5

- Lot 1 (Experimental), consisting of 8 rabbits (5 females and 3 males).

- Lot 2 (Witness), consisting of 8 rabbits (5 females and 3 males);

In the first week of the experimental period, the 5th week of the experiment, but also the 10th week, the animals were subjected to blood samples. Thus, 0.25 ml of blood was collected by puncturing the auricular vein. These samples were taken in heparin tubes and stored for laboratory determinations.

Taking blood samples is a potentially stressful job.

Consequently, it was carried out by the veterinary staff, who supervise the rabbit herd on a daily basis. The above-mentioned staff is used to the animals in the centre and, consequently, the stress following the collection of blood samples is reduced.

Also, given the fact that both batches were subjected to the same potentially stressful manoeuvres, the level of stress induced being similar, we consider the comparisons between the two batches valid.

The blood samples were used for the purpose of evaluating the following haematological parameters: haemoglobin, haematocrit, number of red blood cells, number of leukocytes, as well as the weight of neutrophils and lymphocytes within the leukocyte formula. In order to perform haematological determinations, an IDEXX ProCyte Dx Hematology Analyzer was used.

Children and the experimental design

The study involved 10 children (6 boys and 4 girls) who were recruited from the School Centre for Inclusive Education (C.S.E.I.) "LACRIMA" Bistrița. The ages of the children involved in the study ranged from 7 to 13 years. These children are diagnosed with: psychomotor delay; deficient general and specific motor skills; unfixed laterality; spatial-temporal disorientation; non-development of articulate verbal language; affective instability, autism spectrum disorders (ASD), based on specialised psychiatric and psychological examinations. ASD is characterised by dysfunctional behaviours, communication difficulties, and social interaction (21). The families of these children were notified about the development of this experiment within the C.S.E.I. and expressed their agreement, in writing, for the children's participation in our approach.

The 10 children were separated into two batches:

-Batch 1 (n=5), consisting of children undergoing Rabbit Assisted Therapy (TAI);

-Batch 2 (n=5) consisting of children who were not subjected to Rabbit Assisted Therapy (TAI).

Children from Batch 2 were subjected, for 10 weeks, with a duration of 2 hours with a break of 10 min. (10.00-12.00), with a frequency of 2 sessions per week, to regular treatment sessions (without the use of rabbits), in the presence of a psychotherapist.

The measurements were made with the help of:

1. Aberrant Behaviour Checklist-Community Edition (ABC-C) which measures a variety of challenging behaviours that may be associated with ASD. It is a checklist of 58 items, with five subscales: irritability, lethargy, stereotypy, hyperactivity, and inappropriate speech. ABC-C uses a 4-point rating scale (0-3), with higher scores signifying more problematic behaviours. Its subscales are self-contained, as the manual does not recommend the use of the overall ABC-C score, and each subscale has high internal consistency, recognised validity, and sufficient reliability (3). ABC-C has been shown to be an effective measure of the effects of medications and psychosocial treatment, and its factor structure is robust and valid in ASD. ABC-C has been recommended for use in minimally verbal children with ASD. Pre- and post-ABC-C tests were completed for all participants

before and after a 10-week period. These were completed for each participant.

2. The Pet Intervention Checklist (MOPI) measurement is a four-item observational measure that assesses participants' involvement in an AAI, through attention, physical movement, verbal communication, and compliance. It uses a seven-point Likert scale, with (31) indicating no evidence, (8) for strong evidence of behaviour, and includes a short section for qualitative comments about the participant. It was completed after each intervention session for each participant, according to Table 2.

The order in which the participating children were monitored was randomised and established before the start of each session. Once the data was collected for one child, monitoring of the next child began, until all five children were observed. Once the entire sample was monitored, a second observation period was triggered, with another randomized order of participants. This pattern continued throughout the intervention session.

Statistical analysis

The data obtained were statistically analysed, calculating the Mean and Standard Deviation (SD) using the Microsoft Excel application. At the same time, the statistical significance of the differences between the batches was calculated using the t-test (Student), using the Microsoft Excel application.

RESULTS AND DISCUSSIONS

Rabbit results

The analysis of the blood count values in the case of the two experimental batches prepared during our experiment is centralised in Table 3. The analysis of the leukogram values in the case of the two experimental batches prepared during our experiment is centralised in Table 4. Regarding the haematological examinations, in the case of rabbits from the Experimental batch, the level of the number of red blood cells in the blood was 14.81% higher at the second collection compared to the one recorded in the card of the first collection, the differences being statistically significant ($P < 0.05$) (Fig. 1). Regarding the same batch, we can emphasise that the number of red blood cells identified at the first collection does not differ significantly from the one recorded in the case of the third blood sample.

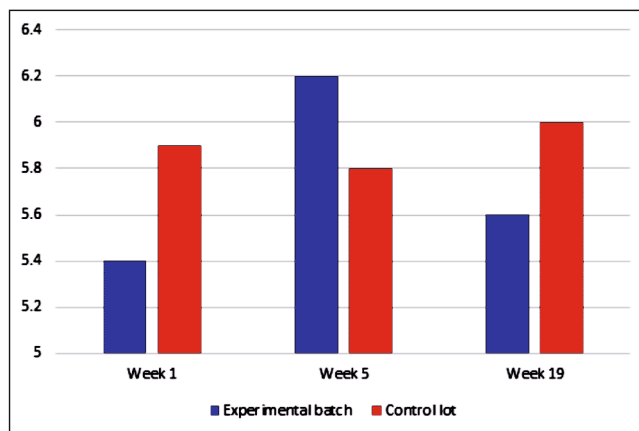


Fig. 1. Average number of red blood cells (M/mL) in blood, in the case of animals from the two experimental groups

In the case of the Experimental batch, the haematocrit level was 13.33% higher at the second collection, compared to the one recorded in the first determination card, the differences being statistically significant ($P < 0.05$) (Fig. 2). Regarding the same batch, we emphasise that the number of red blood cells identified at the first collection does not differ significantly from the one recorded in the case of the third collection.

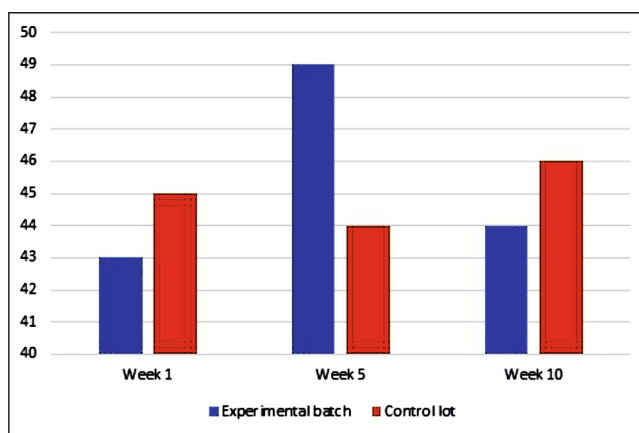


Fig. 2. Average haematocrit (%) in the case of animals from the two experimental groups

In the case of the Experimental group, the haemoglobin level was 10.20% higher at the second collection, compared to the one recorded in the card of

Table 2

Interpretation of the Pet Intervention Checklist (MOPI)

SKILLS	DEFINITION
Attention in the activity	It refers to attention and concentration, as well as the time spent on task (time-on-task) for a certain activity; in other words, the time dedicated to an activity until the beneficiary is distracted.
Physical movements	Fine and gross motor skills (intentional, load-oriented movements, etc.)
Communicate	It refers to verbalisation and observing the client's intention to communicate
Compliance	ability to complete certain tasks.

the first determination, the differences being statistically significant ($P<0.05$) (Fig. 3). Regarding the same batch, we found that the number of red blood cells identified at the first collection does not differ significantly from the one recorded in the case of the third determination.

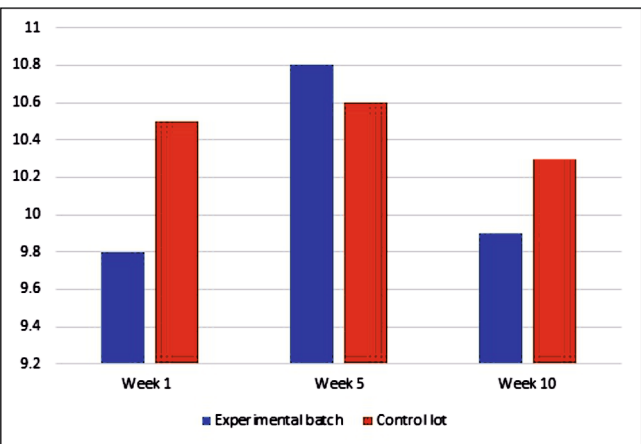


Fig. 3. Average haemoglobin (g/dL) in the case of animals from the two experimental groups

In the case of the Control group, no significant differences were found between the values of the above-mentioned parameters (Red Blood Cells, Haematocrit,

and Haemoglobin), obtained during the three experimental samples ($P>0.05$). In the case of the Experimental batch, the evolution of the three parameters is in direct reciprocal correlation, indicating a slight polycythaemia, a direct consequence of the initial contact between the rabbits used in the experiment and the treated children. Thus, in the first part of the experiment, the initial contact generated a potentially stressful condition on the animals. The sympathocutaneous specific to such a condition initiates an increase in the number of red blood cells, without, however, leading to an exceedance of the normal maximum values.

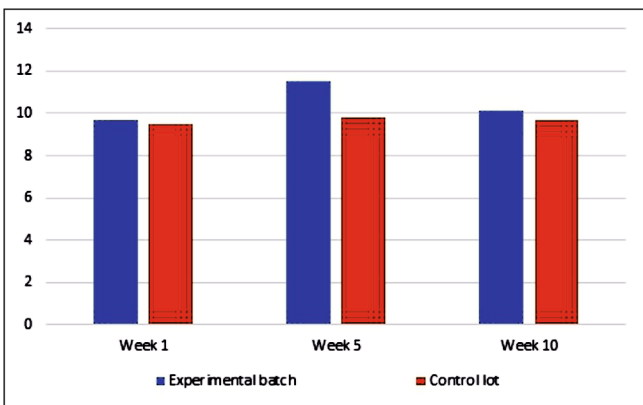


Fig. 4. Weight of Leukocytes (k/mL) in the case of animals from the two experimental groups

Table 3

Blood count values for the two experimental groups

Lot	Average number of red blood cells (M/mL) Average ±SD	Average haematocrit (%) Average ±SD	Average haemoglobin (g/dl) Average ±SD
In week 1 of the experiment			
1. Experimental (n=8)	5.4±0.41	43±4.8	9.8±0.49
2. Control (n=8)	5.9±0.42	45±4.2	10.5±0.5
In week 5 of the experiment			
1. Experimental (n=8)	6.2*±0.22	49*±3.2	10.8*±0.32
2. Control (n=8)	5.8±0.32	44±3.2	10.6±0.32
In week 10 of the experiment			
1. Experimental (n=8)	5.6±0.52	44±4.1	9.9±0.22
2. Control (n=8)	6.0±0.41	46±4.8	10.3±0.30

* $P<0.05$

Table 4

Leukogram values in the case of the two experimental groups

Lot	Leukocyte (K/mL) Average ±SD	Neutrophils (%) Average ±SD	Lymphocytes (%) Average ±SD
In week 1 of the experiment			
1. Experimental (n=8)	9.7±0.34	69±4.8	32±2.2
2. Control (n=8)	9.5±0.25	65±5.1	33±3.2
In week 5 of the experiment			
1. Experimental (n=8)	11.5*±0.29	75*±5.6	25*±3.4
2. Control (n=8)	9.8±0.35	68±5.8	34±4.1
In week 10 of the experiment			
1. Experimental (n=8)	10.1±0.32	69±5.9	33±3.9
2. Control (n=8)	9.7±0.41	70±6.1	35±2.3

* $P<0.05$

As the experiment progressed, as a result of the animals getting used to the children under treatment, there was a decrease in the level of stress developed by the animals used in the experiment.

Regarding the haematological examinations, in the case of rabbits from the Experimental batch, the level of leukocyte count was 18.55% higher at the second collection, compared to the one recorded in the card of the first collection, the differences being statistically significant ($P < 0.05$) (Fig. 4). Regarding the same batch, we can emphasise the fact that the number of leukocytes identified at the first collection does not differ significantly from the one recorded in the case of the third blood sample.

In the case of the Experimental group, the neutrophil share was higher by 8.69% at the second collection, compared to that recorded in the first determination, the differences being statistically significant ($P < 0.05$) (Fig. 5). Regarding the same batch, we emphasise that the neutrophil share identified at the first collection does not differ significantly from that recorded at the third collection.

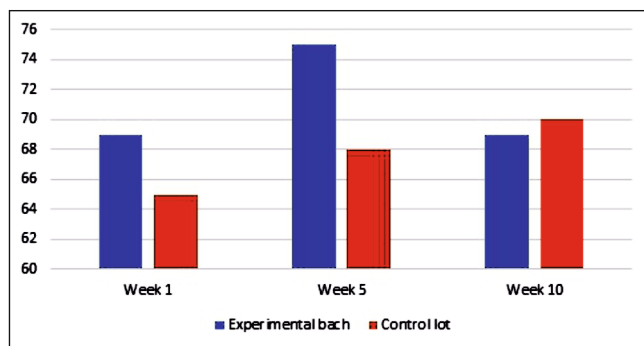


Fig. 5. Share of Neutrophils (%) in the case of animals from the two experimental groups

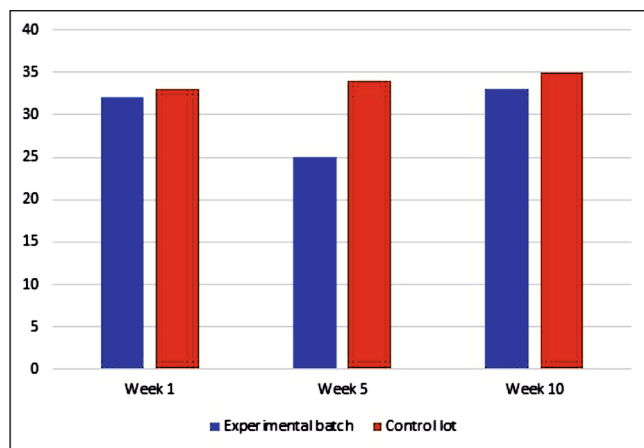


Fig. 6. Share of Lymphocytes (%) in the case of animals from the two experimental groups

In the case of the Experimental group, the share of lymphocytes was lower by approximately 21.87% at the second collection, compared to that recorded in the first determination, the differences being statistically significant ($P < 0.05$) (Table 4).

Regarding the same batch, we found that the share of lymphocytes identified at the first collection does not differ significantly from the one recorded in the case of the third determination. In the case of the Control group, no significant differences were found between the values of the above-mentioned parameters (Leukocytes, Neutrophils, and Lymphocytes), obtained during the three experimental samples ($P > 0.05$).

Dogs and horses are the most common species used in animal-assisted therapy (AAT), but rabbits could also be used in complementary therapy due to their popularity, especially among children (17). In many European countries, rabbits are common pets. Rabbits are present in children's literature as friendly characters, which arouse pleasant emotions among students.

Molnár et al. (2015) (30) showed that interventions with the help of rabbits can reduce anxiety in primary school children, which can have a beneficial impact on teachers' pedagogical performance. Rabbits have been used in therapies in hospitals, nursing homes, veterans' centres, social institutions, kindergartens, and schools, although only a few species and individuals are suitable for such therapeutic interventions (29). Thus, it is essential to have a prior understanding of the animal's behaviour to guarantee the safety of the person involved in the therapy and to ensure the welfare of the animals. It is essential to have information on the stress tolerance capacity of rabbits during sittings, as well as the effect of repeated sittings. This knowledge is important for understanding the methodology of rabbit-assisted intervention, preventing animal burnout, and protecting their mental and physical health (45). Our results indicate the establishment of a potentially stressful condition in the first part of the experiment as a result of the initial contact between the rabbits used in the experiment and the treated children. This evolution was induced by the amplification of the blood level of glucocorticoid hormones. These hormones induce the centralisation of neutrophils, respectively inhibiting their adhesion to the vascular endothelium and delaying diapedesis.

The result is an increase in the total number of leukocytes in the blood fluid. Also, the increase in the blood level of glucocorticoid hormones leads to tissue sequestration of lymphocytes and a decrease in their share in the leukocyte formula. As the experiment progressed, as a result of the animals getting used to the children under treatment, there was a decrease in the level of stress developed by the animals used in the experiment. The results of our experiment highlight the fact that the use of rabbits as auxiliaries in the therapy of autism spectrum disorders in children determined a transient polycythaemia, which diminished by the end of the experiment, supporting a possible eustress to which the animals were subjected only in the first part of the experiment.

The initial and transient eustress is also supported by the evolution of leukocyte parameters, which indicated a slight leukocytosis, neutrophilia, and lymphopenia, manifesting only in the first part of the experiment.

Table 5

**Analysis of the effectiveness of the rabbit intervention
compared to the control group (ABC-C)**

Test measure	Experimental group (n=8)			Control batch (n=8)		
	Pre-test Average $\pm$ SD	Post test Average $\pm$ SD	Change pre-post test	Pre-test Average $\pm$ SD	Post test Average $\pm$ SD	Change pre-post test
Hyperactivity	3.8 $\pm$ 0.21	2.5 $\pm$ 0.21	1.3 $\pm$ 0.10	3.6 $\pm$ 0.01	1.7 $\pm$ 0.11	1.9* $\pm$ 0.05
Lethargy	3.6 $\pm$ 0.22	2.4 $\pm$ 0.18	1.2 $\pm$ 0.05	3.7 $\pm$ 0.07	1.8 $\pm$ 0.21	1.9* $\pm$ 0.06
Stereotypes	3.3 $\pm$ 0.12	2.6 $\pm$ 0.16	0.7 $\pm$ 0.07	3.6 $\pm$ 0.21	1.5 $\pm$ 0.01	2.1* $\pm$ 0.21
Inappropriate speech	3.6 $\pm$ 0.15	2.2 $\pm$ 0.17	1.4 $\pm$ 0.08	3.5 $\pm$ 0.11	1.8 $\pm$ 0.32	1.7* $\pm$ 0.11

*P<0.05

Children's results

The analysis of the measurements made with the help of the aberrant behaviour checklist-Community Edition (ABC-C), in the case of the two experimental batches prepared during the conduct of our experiment, is centralised in Table 5. The analysis of the measurements made with the help of the Pet Intervention Checklist (MOPI), in the case of the two experimental batches drawn up during the conduct of our experiment, is centralised in Table 6. Regarding the hyperactivity of the children involved in the experiment, we found a significant difference between the two groups (P<0.05). In the case of Lot 1 (children who benefitted from TAI), a decrease of 31.5%, compared to Lot 2 (children who did not benefit from (TAI) (Fig. 7).

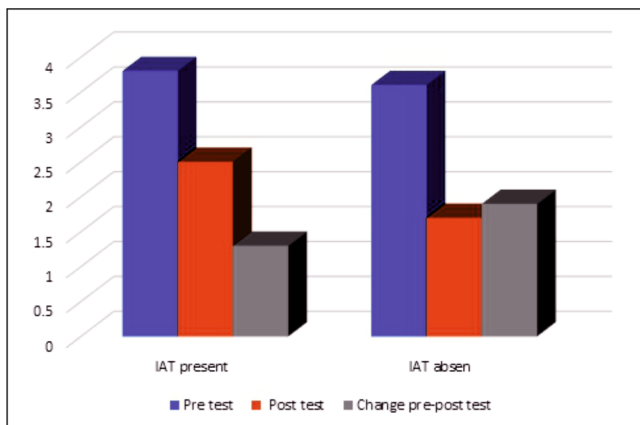


Fig. 7. The percentage weight of the Hyperactivity taken in the study in the case of the two experimental groups

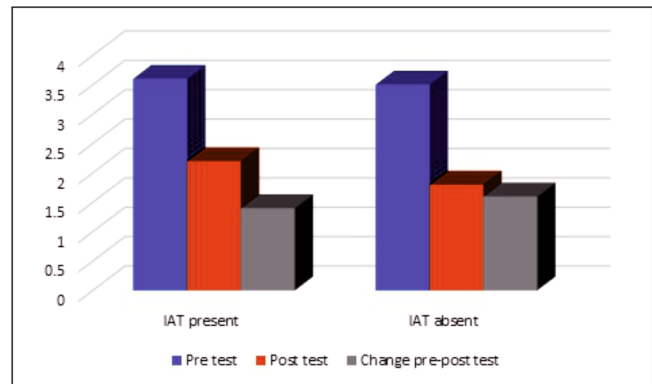


Fig. 8. The percentage share of inappropriate Speech taken in the study, in the case of the two experimental groups

Regarding the inappropriate speech of the children involved in the experiment, we found a significant difference between the two groups (P<0.05). In the case of Lot 1 (children who benefitted from TAI), a decrease of 21.42% compared to Lot 2 (children who did not benefit from TAI) (Fig. 8). Regarding the phenomenon of Lethargy in the children involved in the experiment, we found a difference between the two groups (P<0.05). In the case of Lot 1 (children who benefitted from TAI), a decrease of 22.33% compared to Lot 2 (children who did not benefit from TAI) (Fig. 9). Regarding the stereotypes of the children involved in the experiment, we found a significant difference between the two groups (P<0.05). In the case of Lot 1 (children who benefitted from TAI) a decrease of 66.66% compared to Lot 2 (children who did not benefit from TAI) (Fig. 10).

Table 6

**Analysis of the effectiveness in the intervention of rabbits
compared to the control group (MOPI)**

Test measure	Experimental group (n=8)			Control batch (n=8)		
	Pre-test Average $\pm$ SD	Post test Average $\pm$ SD	Change pre-post test	Pre-test Average $\pm$ SD	Post test Average $\pm$ SD	Change pre-post test
Attention in the activity	2.5 $\pm$ 0.41	5.8 $\pm$ 0.11	3.3 $\pm$ 0.21	2.4 $\pm$ 0.09	6.7 $\pm$ 0.21	4.3* $\pm$ 0.4
Physical movements	3.1 $\pm$ 0.31	5.7 $\pm$ 0.01	2.6 $\pm$ 0.22	3.2 $\pm$ 0.22	6.7 $\pm$ 0.11	3.5* $\pm$ 0.23
Communication	3.6 $\pm$ 0.22	5.2 $\pm$ 0.21	1.6 $\pm$ 0.25	3.1 $\pm$ 0.23	6.2 $\pm$ 0.15	3.1* $\pm$ 0.17
Compliance	3.2 $\pm$ 0.11	5.1 $\pm$ 0.21	1.9 $\pm$ 0.05	3.6 $\pm$ 0.28	6.6 $\pm$ 0.17	3.0* $\pm$ 0.18

*P<0,05

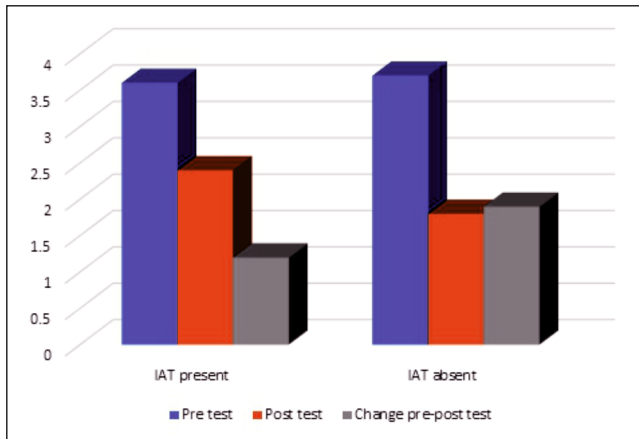


Fig. 9. The percentage weight of the Lethargy phenomenon studied, in the case of the two experimental groups

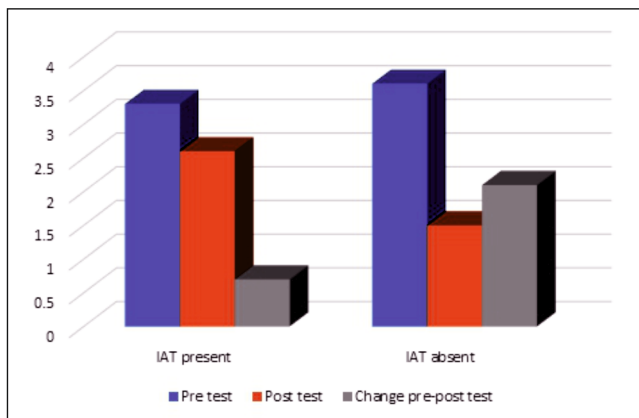


Fig. 10. The percentage weight of the Stereotypes studied in the case of the two experimental groups

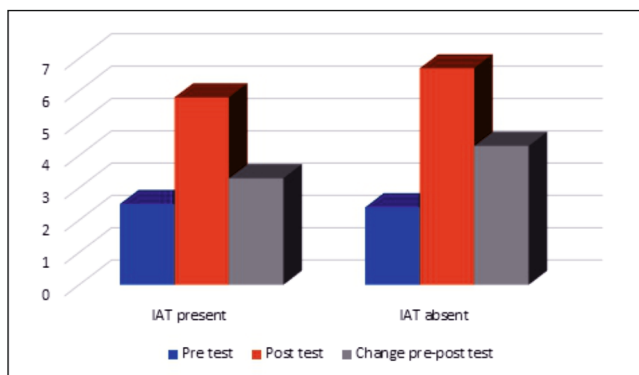


Fig. 11. Percentage share of Attention in the activity (taken in the study) in the case of the two experimental groups

The results regarding the efficacy of the auxiliary therapeutic intervention with rabbits on patients, evaluated by the ABC-C method, are presented in Table 5. Here there are significant differences ($P < 0.05$) between the two lots. We found a drastic decrease in Hyperactivity (31.5%) and Inappropriate Speech (21.42%). The biggest difference was found in the case of

Stereotypes, where the difference was 66.66%. Regarding the Attention in Activity of the children involved in the experiment, we found a significant difference between the two groups ($P < 0.05$). In the case of Lot 1 (children who benefitted from TAI), a decrease of 30.30% compared to Lot 2 (children who did not benefit from TAI) (Fig. 11).

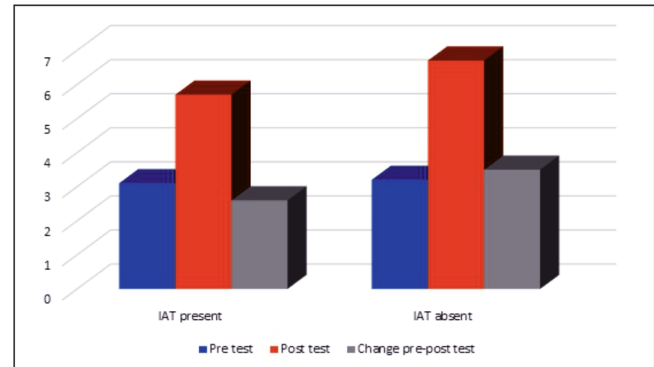


Fig. 12. The percentage weight of the physical movements taken in the study in the case of the two experimental groups

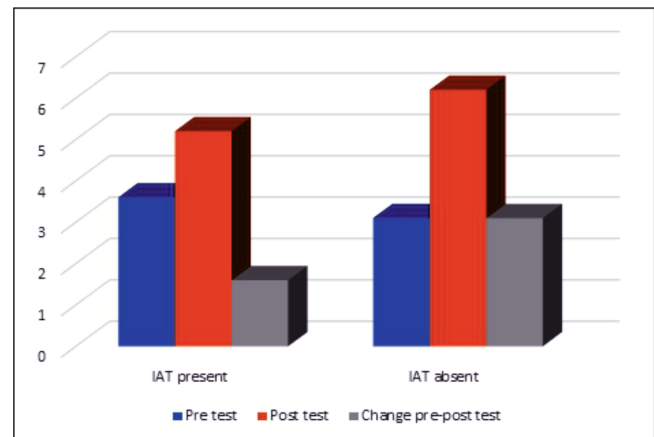


Fig. 13. The percentage weight of the Communication taken in the study in the case of the two experimental groups

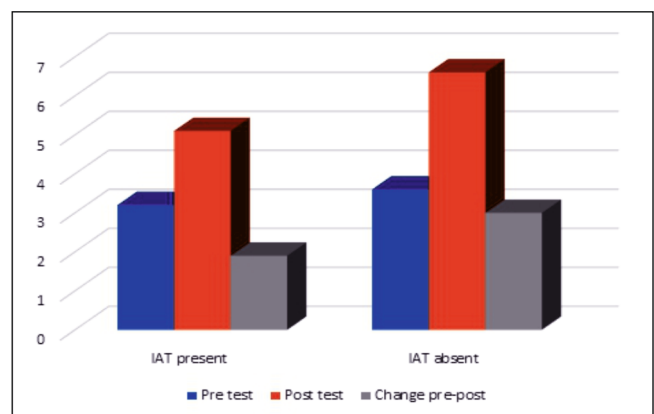


Fig. 14. The percentage weight of the Compliance taken in the study, in the case of the two experimental lots

Regarding the physical movement of the children involved in the experiment, we found a significant difference between the two groups ($P < 0.05$). In the case of Lot 1 (children who benefitted from TAI), a decrease of 23.75% compared to Lot 2 (children who did not benefit from TAI) (Fig. 12).

Regarding the communication of the children involved in the experiment, we found a significant difference between the two groups ($P < 0.05$). In the case of Lot 1 (children who benefited from TAI), a decrease of 93.75% compared to Lot 2 (children who did not benefit from TAI) (Fig. 11).

Regarding the Compliance of the children involved in the experiment, we found a significant difference between the two groups ($P < 0.05$). In the case of Lot 1 (children who benefited from TAI), a decrease of 57.80% compared to Lot 2 (children who did not benefit from TAI) (Fig. 12).

The results on the efficacy of the auxiliary therapeutic intervention with rabbits on patients, assessed by the MOPI method, are presented in Table 6. Here there are significant differences ($P < 0.05$) between the two lots. We found an improvement in Attention (30.30%) and Compliance (57.80%). The biggest difference was found in the case of the Communication, where the difference was 93.75%. Our results strongly support the fact that the auxiliary therapy of autism spectrum disorders with the help of rabbits is an effective manner of intervention, which can bring substantial improvements in therapeutic outcomes. From our research, it follows that the association of TAI with current therapy of children with ASD left a significant imprint on the parameters highlighted in our study. Our observations on the effect of TAI on hyperactivity indicate a decrease. It also cost a drastic decrease in the case of stereotypes, where the difference was 66.66%.

Our results are consistent with those obtained by Deanna et al. (2022) (46) indicating a positive influence on the affective status of the children involved in the experiment. In our research, we also had among the working hypotheses the idea that interaction with animals, during treatment sessions against autism spectrum disorders, should also improve communication. We believe that this may be a consequence of contact with animals and a result of the exercises done by the child together with the rabbits in the experiment. We also highlight the positive effect of the TAI on the parameters related to attention in activity and compliance, noting a consistent improvement. In our analysis, we should highlight the high weight (93.75%) in the case of communication shown by the children in the experimental group, representing a nine times higher level, compared to the observations regarding the children in the control group. Our research highlights the positive effects of rabbit-supported approaches on children with autism. Here we note, especially with regard to sensory, emotional, and physical functioning, findings that are in line with the results published by Chin-Siang Ang et al. (2022) (4).

The objective of our research did not include assessing whether the use of rabbits in therapy had a cal-

ming effect on children. Therefore, we believe that it would be beneficial to deepen our research in this field of study in the future. We also suggest further research investigating the effect of rabbit therapy on physical movements. Last but not least, we emphasise that it would be important to analyse the effect on restrictive and repetitive behaviours.

In this case, the authors explained these results by a potential calming effect of rabbit therapy on children with ASD, associating in this direction the research carried out by Kirnan et al. (2018) (26), who highlighted such an effect in the presence of dogs. Coming to this discussion, we should note that not all research has highlighted a calming effect of therapy associated with rabbits, in the case of children with autism spectrum disorders. The results reported by Germone et al. (2019) (22), who highlighted an increase in the level of agitation in the presence of treatment dogs in children with autism spectrum disorders, should perhaps be deepened. However, our study found that TAI is not a treatment that ensures the reintegration of children, but only a way that can help alleviate some symptoms associated with ASD. Our results strongly support the fact that the auxiliary therapy of autism spectrum disorders with the help of rabbits is an effective manner of intervention, which can bring substantial improvements in therapeutic results. Our research entitles us to argue that further qualitative and quantitative research is needed, and that more programs are also needed to focus on the therapeutic use of animals in children's therapy. These positive results, according to our findings, do not hinder the level of welfare of the animals used in the therapy.

CONCLUSIONS

The data provided by the ethograms resulting from our experiment indicated an increase in stress-related behaviours in rabbits used in therapy. The specific activities of rabbit-assisted therapy, in the case of children with autism spectrum disorders, determined a transient polycythaemia, which diminished until the end of the experiment, supporting a possible eustress to which the animals were subjected, only in the first part of the experiment. The initial and transient eustress is also supported by the evolution of leukocyte parameters, which indicated a slight leukocytosis, neutrophilia, and lymphopenia, manifesting only in the first part of the experiment. The use of rabbits as auxiliaries in the therapy of autism spectrum disorders in children had positive effects on therapeutic efficiency. This is underlined by the results of both tests used in our experiment (ABC-C and MOPI), which revealed improvement in specific symptomatology. We can emphasise that this type of therapy does not result in amplifying the level of stress to which used animals are exposed. These results encourage the further application of these therapeutic methods, which have no negative effect on the welfare of the participating animals.

REFERENCES

1. Abelson A.G., (1999), Respite care needs of parents of children with developmental disabilities. Focus on Autism and Other Developmental Disabilities, 14(1999):96-100
2. Aman M.G., Novotny S., Samango-Sprouse C., Lecavalier L., Leonard E., Gadow K., (2004), Outcome measures for clinical drug trials in autism. CNS Spectr, 9:36-47
3. Aman M.G., Singh N.N., Stewart A.W., Field C.J., (1985), The Aberrant Behavior Checklist: A behavior rating scale for the assessment of treatment effects. Am J Ment Defic, 89:485-491
4. Ang, C. S., MacDougall F.A., (2022), An Evaluation of Animal-Assisted Therapy for Autism Spectrum Disorders: Therapist and Parent Perspectives. Psychological studies, 67(1):72-81
5. Asselin G., Penning J.H., Ramanujam S., Neri R., Ward C., (2012), Therapeutic horseback riding of a spinal cord injured veteran: a case study. Rehabil Nurs, 37:270-276
6. Baard M., van der Elst D., lastig O., (2006), Inzicht in veel voorkomende ontwikkelingsstoornissen bij kinderen (Unintentionally Troublesome: Insight in Many Frequently Occurring Pervasive Developmental Disorders in Children), (Ed.) Pica, Huizen, Nederland
7. Bartak L., Rutter M., (1973), Special educational treatment of autistic children: a comparative study 1. Design of study and characteristics of units. Journal of Child Psychology and Psychiatry and Allied Disciplines, 14:161-179
8. Bass M.M., Duchowny C.A., Llabre M.M., (2009), The effect of therapeutic horseback riding on social functioning in children with autism. J Autism Dev Disord, 39:1261-1267
9. Boyd B.A., (1999), Examining the relationship between stress and lack of social support in mothers and children with autism. Focus on Autism and Other Developmental Disabilities, 17(2002):208-215
10. Brakman E., (2005), Jeugdhulpverlening op zorgboerderijen? Natuurlijk! (Youth Care at Green Care farms? Naturally!), Stichting Stimuland Overijssel, Ommen, Nederland
11. Broom D.M., Fraser A.F., (2015), Domestic Animal Behaviour and Welfare, 5th ed., (Ed.) CABI Wallingford, Wallingford, UK, 2015, 462
12. Campbell M.L., Sandøe P., (2015), Welfare in horse breeding. Vet Rec, 176(17):436-440
13. Chan J.B., Sigafos J., (2000), A review of child and family characteristics related to the use of respite care in developmental disability services. Child and Youth Care Forum, 29(2000):27-37
14. Chan J.B., Sigafos J., (2001), Does respite care reduce parental stress in families with developmentally disabled children? Child and Youth Care Forum, 30(2001):253-263
15. Cotor G., Zagrai G., Gâjăilă G., Ghiță M., Ionescu A.M., Damian A., Zagrai Măierean A.M., Dragosloveanu Ș., Cotor D.C., (2021), The evolution of some blood parameters in hypovolemia conditions in rabbits. Pol J Vet Sci, 24(4):589-594
16. Cunningham J.G., (2002), Textbook of Veterinary Physiology, 3rd ed., (Ed.) W.B. Saunders Company (Elsevier), Philadelphia, USA
17. Demello M., (2016), Rabbits multiplying like rabbits: The rise in the worldwide popularity of rabbits as pets. Companion Anim Everyday Life, 2016:91-107
18. Despoupoulos A., Silbernagl S., (2003), Color Atlas of Physiology, (Ed.), Thieme, Stuttgart, Germany
19. Didden R., Huskens B. (Eds.), (2008), Begeleiding van kinderen en jongeren met autisme: Van onderzoek naar praktijk (Accompaniment of Children and Youngsters with Autism: From Research to Practice), (Ed.) Bohn Stafleu van Loghum, Houten, Nederland
20. Fine A.H., (2011), Our ethical and moral responsibility: Ensuring the welfare of therapy animals foundations and guidelines for animal-assisted interventions. In: Handbook on Animal-Assisted Therapy, 4th ed, (Ed.) Academic Press, Cambridge, MA, USA, 357-376
21. Frye R.E., Rossignol D.A., (2016), Identification and treatment of pathophysiological comorbidities of autism spectrum disorder to achieve optimal outcomes. Clin Med Insights Pediatr, 10:43-56
22. Germone M.M., Gabriels R.L., Guérin N.A., Pan Z., Banks T., O'Haire M.E., (2019), Animal-assisted activity improves social behaviors in psychiatrically hospitalized youth with autism. Autism, 23(7):1740-1751.
23. Jones G., (2006), Department for Education and Skills & Department of Health Good Practice Guidance on the education of children with autistic spectrum disorder. Child Care Health and Development, 32:543-552
24. Kaat A.J., Lecavalier L., Aman M.G., (2014), Validity of the Aberrant Behavior Checklist in children with autism spectrum disorder. J Autism Dev Disord, 44:1103-1116
25. Kasari C., Brady N., Lord C., Tager-Flusberg H., (2013) Assessing the minimally verbal school-aged child with autism spectrum disorder. Autism Res, 6:479-493
26. Kirnan J., Shah S., Lauletta C., (2018), A dog-assisted reading programme's unanticipated impact in a special education classroom. Educational Review, 72:196-219
27. Koolhaas J.M., Bartolomucci A., Buwalda B., de Boer S.F., Flügge G., Korte S.M., Meerlo P., Murison R., Olivier B., Palanza P., Richter-Levin G., Sgoifo A., Steimer T., Stiedl O., van Dijk G., Wöhr M., Fuchs E., (2011), Stress revisited: a critical evaluation of the stress concept. Neurosci Biobehav Rev, 35(5):1291-1301
28. Loukaki K., Koukoutsakis P., Kostomitsopoulos N., (2010), Animal welfare issues on the use of rabbits in an animal assisted therapy program for children. J Hell Vet Med Soc, 61:220-225
29. Molnár M., Iváncsik R., Di Blasio B., Nagy I.,

- (2020), Examining the effects of rabbit-assisted interventions in the classroom environment. *Animals*, 10:26
30. Molnár M., Rudolf Z., Szalai K., Takács I., (2015), Pedagógusképzést segítő hálózatok továbbfejlesztése a Dél-Dunántúl régióban (in Hungarian), In: Állatasszisztált pedagógiai és terápia, (Ed.) LPI Publisher, Budapest, Hungary
 31. Oikawa M., Hobo S., Oyamada T., Yoshikawa H., (2005), Effects of orientation, intermittent rest and vehicle cleaning during transport on development of transport-related respiratory disease in horses. *J Comp Pathol*, 132:153-168
 32. Paulev P.E., (2000). Textbook in Medical Physiology and Pathophysiology, Essentials and clinical problems, Copenhagen Medical Publishers, Copenhagen, Denmark
 33. Peeters M., Sulon J., Beckers J. F., Ledoux D., Vandenheede M., (2011), Comparison between blood serum and salivary cortisol concentrations in horses using an adrenocorticotrophic hormone challenge. *Equine vet. journal*, 43(4):487-493
 34. Popescu S., Diugan E.A., Spinu M., (2014), The interrelations of good welfare indicators assessed in working horses and their relationships with the type of work. *Res Vet Sci*, 96(2):406-414
 35. Popescu S., Lazar E.A., Borda C., Niculae M., Sandru C.D., Spinu M., (2019), Welfare quality of breeding horses under different housing conditions. *Animals (Basel)*, 9(3):81
 36. Preziosi R.J., (1997), For your consideration: A pet - assisted therapy facilitator code of ethics. *Latham Lett*, 18:5-8
 37. Rodrigue J.R., Morgan S.B., Geffken G.R., (1990), Families of autistic children: psychological functioning of mothers. *Journal of Clinical Child & Adolescent Psychology*, 19:371-379
 38. Ruble L.A., McGrew J.H., (2007), Community services outcomes for families and children with autism spectrum disorders. *Research in Autism Spectrum Disorders*, 1 (2007):360-372
 39. Rutter M., Bartak L., (1973), Special educational treatment of autistic children: a comparative study. 2. Follow-up findings and implications for services. *Journal of Child Psychology and Psychiatry and Allied Disciplines*, 14:241-270
 40. Salak-Johnson J., McGlone J., (2006) Making sense of apparently conflicting data: Stress and immunity in swine and cattle. *J Anim Sci*, 85:E81-E88
 41. Sanders J.L., Morgan S.B., (1997), Family stress and adjustment as perceived by parents of children with autism or Down syndrome: implications for intervention. *Child and Family Behaviour Therapy*, 19:15-32
 42. Schiro-Geist C., Heimlich K., (1997), Evaluating the effects of animal-assisted therapy on severely disabled children, In: Proceedings of the Delta Society Sixteenth Annual Continuing Education and Training Conference, 17-19 October 1997, Atlanta, GA, USA
 43. Schopler E., Brehm S.S., Kinsbourne M., Reichler R.J., (1971), Effect of treatment structure on development in autistic children. *Archives of General Psychiatry*, 24:415-421
 44. Sharpley C.F., Bitsika V., (1997), Efremidis, Influence of gender, parental health, and perceived expertise of assistance upon stress, anxiety and depression among parents of children with autism. *Journal of Intellectual and Developmental Disability*, 22:19-28
 45. Suba-Bokodi É., Nagy I., Molnár M., (2022), Changes in the Stress Tolerance of Dwarf Rabbits in Animal-Assisted Interventions. *Applied Sciences*, 12(14):6979
 46. Tepper D.L., Landry O., Howell T.J., Stephens D., Molina J., Bennett P.C., (2022), Therapy dogs for children with autism spectrum disorder: Impacts of active versus passive dog engagement. *Human-Animal Interaction Bulletin*, 13(1):75-90
 47. van der Veen-Mulders L., Serra M., van den Hoofdkamer B.J., Minderaa R.B. (Eds.), (2005), Sociaal onhandig: De opvoeding van kinderen met PDDN OS en ADHD (Social Awkwardness: The Upbringing of Children with PDD-NOS and ADHD), (Ed.) Van Gorcum BV, Assen, Nederland
 48. Vermeulen P., Bedriegt B., (1998), Als autisme niet op autisme lijkt (The Brain Deceives: When Autistic Disorder Doesn't Look Like Autistic Disorder), (Ed.) EPO, Gent, Belgium
 49. Ward P.A., Blanchard R.J., Bolivar V., Brown M.J., Chang F., Herman J.P., Zawistowski S.L., (2008), Recognition and alleviation of distress in laboratory animals, (Ed.) National Academies Press, Washington, DC, USA
 50. Wehman T., (1998), Family-centered early intervention services: factors contributing to increased parent involvement and participation. *Focus on Autism and Other Developmental Disabilities*, 13 (1998):80-86
 51. Williams D., (2003), Exposure Anxiety: The Invisible Cage, (Ed.) Jessica Kingsley Publ., London, UK.