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Physiology and Clinical Pathology of Laboratory New Zealand White Rabbits Housed Individually and in Groups

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Rabbits used in the production of antibodies can be housed individually or in groups. This study compared the serum chemistries, antibody production, physiologic plasma cortisol levels, and white blood cell (WBC) counts of female New Zealand White rabbits housed in 2 different housing systems. The control group was housed individually in stainless steel cages, and the experimental group was group-housed on aspen shavings spread on the floor of the animal room. Plastic crates were placed in the group-housing area to provide opportunities for rabbits to hide, and a litter box was available at all times. Both groups received the same food and water and similar environmental enrichment devices. Clinical pathology laboratory evaluations of serum chemistries, immune responses, physiologic parameters such as plasma cortisol, and WBC counts were compared. The group-housed animals had lower WBC counts and higher levels of plasma cortisol than did rabbits individually housed. In addition, the group-housed animals had significantly less weight gain during the first week. Antibody production did not differ between the 2 groups. Group housing appeared to be an appropriate method of housing rabbits for use in research.

Abbreviation: WBC, white blood cell count

The use of New Zealand White rabbits (*Oryctolagus cuniculus*) for antibody production is a common procedure in research animal facilities. A widespread practice is to house laboratory rabbits individually in stainless steel cages. Animal Welfare Regulations[1] and the *Guide for the Care and Use of Laboratory Animals*[14] (the *Guide*) have established minimum cage sizes but the *Guide* also states that "cage heights should take into account typical postures of an animal."[14] New Zealand White rabbits frequently sit up on their hind legs when provided with the opportunity. This posture would be impossible for an adult rabbit in a cage 14 in. tall, the minimal allowable height under current animal welfare regulations.

In the wild, rabbits are social animals, exhibiting many social behaviors such as grooming and foraging in groups. [8,13] The *Guide* states that "whenever it is appropriate, social animals should be housed in pairs or groups."[14] However, the limited space and isolation of housing rabbits individually in cages prevent mutual grooming, play, and the erect 'prairie dog' posture that they assume when investigating disturbances.[13]

In addition, individual housing and the resulting lack of movement can lead to physical abnormalities such as osteoporosis and spinal and hip disorders.[2,6,11,15,16] Further, heavy body weight, wire-floored cages, or prolonged contact with urine-soaked bedding are the most important predisposing cases of ulcerative pododermatitis in rabbits.[9] Deformations of the vertebral column in New Zealand White rabbits was dependent on cage size in one study.[6] In another study,[15] laboratory rabbits kept in 'traditional' cages tended to develop stereotypic behaviors and bone deformities.

Group housing of New Zealand White rabbits provides increased opportunities for exercise, social contact, and more natural behavior while accommodating the limitations imposed by experimental conditions.[12,16] Physiologic and immunologic measurements did not differ significantly between single- and group-housed rabbits, indicating that the practical research performance (immune response, stress level, growth rates, and so forth) of these rabbits was not affected by housing type.[16] In addition, the opportunity to exhibit natural behaviors that could increase the research rabbits' quality of life was amenable to manipulation in the research setting through group housing. However, animal care facilities usually single-house rabbits to aid identification, minimize disease spread, make the control and observation of food and water intake easier, and to expedite cleaning and handling.[16]

The objective of the current study was to compare the physiologic parameters and immune responses (including experimentally induced antibody response) of individually housed rabbits with those of animals maintained as a group. Previously published studies[11,13] focused on the social needs of rabbits in a laboratory setting and did not compare antibody titers and physiologic markers of stress between single- and group-housed rabbits, as we do in the current study

Materials and Methods

Subjects. Six female New Zealand White rabbits ranging in weight from 2.0 to 2.5 kg and reported free of *Pasteurella multocida*, *Salmonella* spp., *Treponema cuniculi*, and *Eimeria* spp. were obtained (Western Oregon Rabbits, Philomath, OR).

Each rabbit was uniquely identified with an ear tag. Animals were selected at random and assigned consecutive identification numbers. Animals with the lower identification numbers were assigned to the 'single-housed' group, and animals with higher identification numbers were assigned to the 'group-housed' group. Group-housed rabbits also were identified with nontoxic paint on the head; 3 different colors were used to differentiate

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the rabbits. The paint marking was visible 2 to 3 m from the animal and was reapplied every 2 wk during the 113 d of the study. Animals were exsanguinated and humanly euthanized at the end of the study.

The rabbits were cared for in accordance with federal and local animal welfare regulations in an AAALAC-accredited facility, and the study was approved by the institutional animal care and use committee. All rabbits were provided with a 12:12-h light:dark cycle; room temperature was maintained at 17 to 21 °C; and relative humidity was between 30% and 70%. Both groups had access at all times to water distributed by an automatic watering system (Edstrom, Waterford, WI). The water valves were tested once daily for patency, and the chlorine level was tested every 2 wk to maintain 3 to 6 ppm in the drinking water. All rabbits were fed commercial diet (Hi-Fiber Diet 5326, Purina Mills International, St Louis, MO) daily.

Experimental groups. ***Single-housed group (control).*** On their arrival, 3 female New Zealand White rabbits were single-housed in standard stainless steel cages (16 in. × 24 in. × 24 in.; Lenderking Caging Products, Millersville, MD). The collection pans were cleaned daily with water, and the animals were transferred to a sanitized cage every 2 wk. The 3 rabbits were in a room with other caged rabbits. Each rabbit was fed 120 g of rabbit chow and one handful of timothy hay (Kaytee Products, Chilton, WI) soon after arrival and housing assignment. Thereafter, rabbits received 240 g of chow and 1 handful of timothy hay daily. Twice weekly, they received fruit or vegetables and timothy hay cubes (Bioserv, Frenchtown, NJ) as a supplement to their normal diet. In addition, as part of their environmental enrichment program, they received hard doughnut-shaped treats (fruit-flavored and containing sucrose, maltose, dextrin, and flavoring agents; Bunny Blocks, Bioserv) once each week.

Group-housed rabbits. On their arrival, 3 rabbits were housed together in a room measuring 3.3 m × 2.7 m. No other rabbits were housed in the same room during the experiment. Three inches of Aspen Shavings (Harlan Teklad, Madison, WI) were spread on the floor. Three plastic crates measuring 53 cm × 33 cm × 33 cm were placed within the area providing opportunities for rabbits to hide. A litter box measuring 33 cm × 33 cm × 18 cm was available at all times, was filled with approximately 3 cups of litter (Small Animal Bedding, Fangman Specialties, Cincinnati, OH), and was changed 3 times weekly, when it was completely emptied and refilled. An extension to the watering system provided 3 lixit water valves. One double-weighted food dish was filled with fresh pellets daily (240 g of rabbit chow per animal), except for the day of arrival, when they were fed half of the ration. This dish was cleaned daily with a mild detergent and water and disinfected every 10 to 11 d. Several enrichment devices were placed in the area including, but not limited to, plastic toys, jingle balls, hanging plastic chains, and hard doughnut-shaped treats. The room was cleaned and disinfected every 10 to 11 d, and fresh shavings were set on the floor. Soiled bedding was removed from the area daily; additional shavings were added if the bedding was less than 8 cm deep after removal. The contents of the food bowl were removed and a fresh supply of rabbit food was offered daily. Twice weekly, fresh fruit or vegetables were provided; each day, several timothy hay cubes and 1 hard doughnut-shaped treat were provided per rabbit. The treats were hung on the plastic crates, and the hay cubes were spread on the floor.

Handling and restraint. Prior to antigen administration and blood collection, rabbits were captured by hand from their cage, transported to a procedure room (approximately 2.5 m away), and placed in individual restraint cages. Single-housed animals were grasped by the neck scruff by hand, with the bulk of the animal then cradled in the other arm. This process usually took 5 to 10 s. Group-housed rabbits were captured by gently herding the animal into one of the crates on the floor, after which they then could be picked up as described. This process took usually 1 to 2 min.

Antigen administration. The dorsum between the shoulder blades was shaved and wiped with 70% isopropyl alcohol. All rabbits were immunized subcutaneously with antigen–adjuvant emulsion on day 8. The emulsion was prepared by emulsifying 0.5 ml of antigen (purified HKB11 host cell protein[5] in PBS; concentration, 0.2 to 2.0 mg/ml) with an equal volume of adjuvant (lot number 08/01/06801, Titermax Gold, Titermax, Norcross, GA) in a total volume of no more than 1 ml. Rabbits received a SC booster of antigen (1 mg/ ml) [10] without adjuvant on day 45. All drugs were administered at ambient temperature.

Blood collection. Approximately 10 min prior to blood collection, rabbits were injected SC with 0.75 mg/kg acepromazine, and topical anesthetic cream (lidocaine, 2.5%; prilocaine, 2.5%) was applied to cover collection site. The site of sample collection on the ear was prepared by shaving the area and then wiping with 70% isopropyl alcohol. Once prepared, an angiocath (22-gauge, 1-in., Abbocath, Abbot Medical, Abbot Park, IL) was placed in the auricular (central) artery. A 10-ml blood sample was obtained from each rabbit on day 8 (baseline) and again on days 29 and 60. All samples were analyzed for complete blood count, albumin, albumin:globulin ratio, alkaline phosphatase, alanine aminotransferase, aspartate aminotransferase, bicarbonate, total bilirubin, direct bilirubin, indirect bilirubin, BUN, BUN:creatinine ratio, calcium, cholesterol, creatine kinase, creatinine, globulin, glucose, phosphorus, potassium, total protein, sodium, sodium:potassium ratio, and serum cortisol concentration (IDEXX Laboratories, Sacramento, CA) and antibody titer (Bayer Process Sciences Department).

Weight collection. The rabbits were weighed on arrival (day 1) and then prior to blood collection or immunization with antigen (days 8, 29, 45, and 60), for a total of 5 times during the 113-d study.

Monitoring. All animals were examined daily for injection site reactions, evidence of aggression (group-housed animals), and so on.

Statistical analysis. The mean and SEM were calculated for each of the measured parameters. Differences between the individually and group-housed groups were determined by nonpaired, 2-tailed Student *t* tests. Differences were considered to be statistically significant if the probability of the null hypothesis was less than 0.05 (that is, $P < 0.05$). All statistical analyses were performed by using the JMP statistical software package (version 6.0, SAS, Cary, NC).

Results

Monitoring. After receiving their first doses of antigen, 1 of the group-housed and 2 of the single-housed rabbits developed sterile subcutaneous abscesses and granulomas at the injection site. All 3 rabbits were treated with enrofloxacin (10 mg/kg IM daily for 5 d) and a single dose of buprenorphine (0.03 mg/kg IM), and the lesion was flushed with 2% chlorhexidine solution daily for 5 d. The abscesses in the single-housed rabbits resolved by day 5 of treatment. The group-housed rabbit had not healed by the end of the 5-d regimen, and treatment continued for another 16 d (enrofloxacin [10 mg/kg IM daily] and flushing of the lesion with 2% chlorhexidine solution daily for 16 d). In addition, this rabbit received buprenorphine (0.03 mg/kg SC) daily for 3 d. The abscess resolved after 21 d but recurred after

2 wk, at which time the rabbit was treated for 2 consecutive weeks with enrofloxacin (10 mg/kg IM daily) and a single dose of buprenorphine (0.03 mg/kg SC). The abscess had resolved by the end of the treatment.

No adverse affects, including aggression, were noted among the group-housed rabbits. Group-housed rabbits frequently were seen ambulating, hopping, and interacting with cagemates (Figure 1).

By day 8, the single-housed animals had gained significantly more weight (mean ± SEM, 2.55 ± 0.08 kg) than had the group-housed rabbits (2.40 ± 0.06 kg; Figure 2). However, body weight gain did not differ between groups at any of the subsequent observation points.

WBC counts were higher in single-house rabbits than group-housed rabbits on days 8 and 60 but not on day 29 (Figure 3). No other intergroup differences in hematologic parameters were noted. Rabbits in both groups were lymphopenic (single-housed, 26.00% ± 7.55%; group-housed, 28.33% ± 3.48%) compared with the reference range (43% to 62%, IDEXX Laboratories).

Serum cortisol concentration was higher in group-housed than single-housed rabbits on day 8 but did not differ between groups at any other time point (Figure 4). No other serum chemistry value differed between groups, and all values for both groups were within normal references ranges.

The spectrum of HKB11 host cell protein was unique for each of the 6 antisera. Antibody titer did not differ between the 2 groups of rabbits, according to results from Western blotting[4] and enzyme-linked immunosorbent assays.[7]

Discussion

This study compared single and group housing of New Zealand White rabbits during the generation of antibodies. The parameters compared included hematology, blood chemistry, immune responses, physiologic parameters, and behavioral observations. Compared with single-housed animals, group-housed rabbits had lower WBCs on days 8 and 60, higher serum cortisol concentration on day 8 d, and slower weight gain during the first week. The differences on day 8 in both weight gain and cortisol may both be related to greater activity by animals in the novel, group situation, although activity was not measured. The differences in WBCs are more difficult to explain. Acute stress causes leukopenia in rabbits whereas it causes leukocytosis in other mammals.[3] The stimulation caused by catching the group-housed animals prior to blood collection may therefore have caused transient leukopenia. In addition, several of the animals developed abscesses during this time. However, the frequency of abscesses was low (1 abscess among group-housed animals and 2 in those single-housed), with no significant difference between the 2 groups. Furthermore, antibody titers were not significantly different between the two groups.

From a management perspective, catching the rabbits presented an additional challenge as compared with individual housing in cages. If the rabbits had a hiding place, and if staff members approached them quietly, catching the animals was simplified. Informal questioning indicated that the husbandry technicians found group housing rewarding not only for the rabbits but also for themselves. The staff enjoyed seeing the rabbits exhibiting species-specific behaviors such as hopping around the pen, standing on their hindlimbs, and grooming each other. When animal care personnel were asked whether they felt that cleaning the group-housing room was too labor-intensive as compared with that for the single-housing room, the technicians responded that they did not mind the extra labor as long as the rabbits appeared to be 'happier.'

Figure 1. Rabbits in group-housing environment.

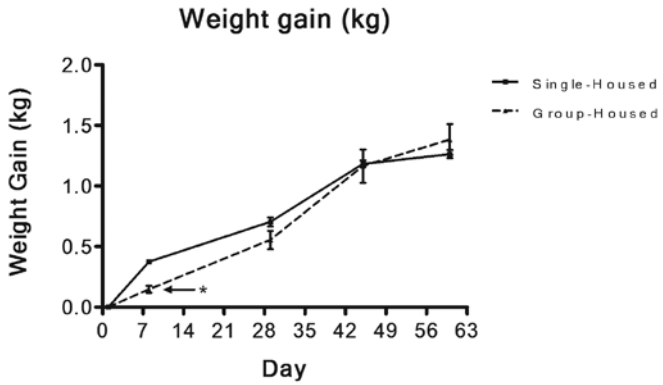

Figure 2. Day 8 body weight was higher (*, $P < 0.05$ [unpaired Student *t* test]) in single-housed rabbits than in group-housed rabbits.

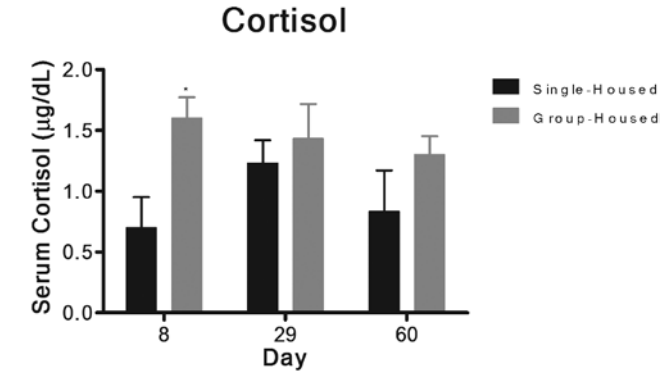

Figure 3. (*, $P < 0.05$ [unpaired Student *t* test]) in group-housed rabbits on days 8 and 60.

Litter boxes were provided within the area but were not always used by the rabbits. Although grossly soiled bedding material was removed from the area daily, a change schedule of every 2 wk was inadequate. A complete room change-out was necessary every 10 to 11 d (depending on occupancy) to provide acceptable sanitation.

The *Guide* and animal welfare regulations encourage housing based on social needs of research animals. The results of this study demonstrate that rabbits used in antibody production can be group-housed practically with no negative effect on the

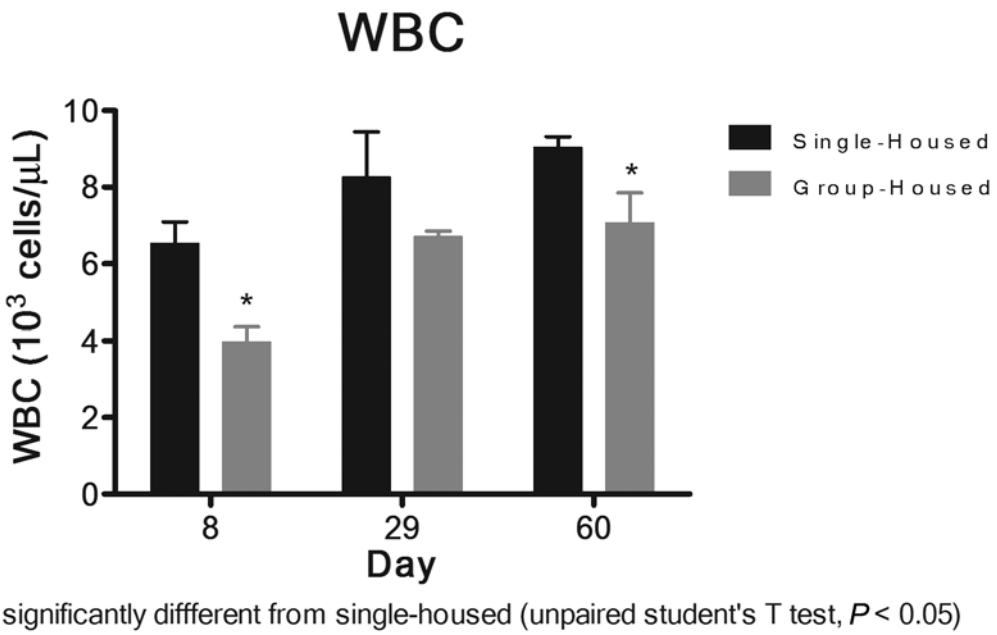

Figure 4. Cortisol level on day 8 was higher (*, $P < 0.05$ [unpaired Student *t* test]) in group-housed rabbits.

research. Group housing should be considered as an approach to housing rabbits for use in research.

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