Influence of different types of bedding materials on immune response and serum biochemical profile of caged mice

Vijayakumar . R, R.Samanta*, A.K.Samanta, R. Guria and S. Joardar**

Department of Livestock Production Management
**Department of Veterinary Microbiology
Faculty of Veterinary & Animal Sciences
West Bengal University of Animal & Fishery Sciences, Kolkata - 700 037
*Corresponding author Email: rajarshisamanta@yahoo.in

Abstract

Contact bedding material is an important environmental factor and welfare creator for laboratory mice. It can alter important physiological process and create potential chance for experimental variation which puts hurdle for comparability. The present experiment was conducted to assess the possible impact of different types of bedding material viz CPS, NWS and PH with fifty one albino mice for a period of fifteen weeks in Laboratory Animal Facility. It was observed that local immunity in mice was enhanced in NWS. During in vitro immune assays, mice from NWS showed higher OD value for reactive oxygen radical, produced more NO2 and higher stimulation index i.e. 0.71 ± 0.01, 30.67 ± 0.88 µM and 7.90 ± 0.17, respectively than PH (0.23 ± 0.01, 17.0 ± 1.15 µM and 6.33 ± 0.21) and CPS (0.33 ± 0.03, 15.67 ± 1.20 µM and 6.46 ± 0.27). There was no influence of bedding type on systemic response. Reduced glutathione value in liver was higher in NWS than PH and CPS i.e. 8.54 ± 0.2, 7.09 ± 0.18 and 6.96 ± 0.14 µmole/ gm of tissue. But heart reduced glutathione showed no variation among different types of bedding materials. Serum analysis showed significantly ($p<0.05$) higher total protein and albumin value for enriched groups. But globulin value was not significantly differing for enriched and non-enriched groups.

Key Words: Contact bedding, Reduced glutathione, Immune response, Mice.

Introduction

Recent studies explore the preferences for various types of bedding and provide cautionary information with regard to some potential health effects and experimental impact of bedding materials. Most important and often bypassed hotspot in laboratory animal management is contact bedding materials provided to the laboratory animals. Bedding material which was commonly used for laboratory animals includes sawdust, wood shavings, dried wood chips, paddy husk and sugar beet (Lane-Petter,1972). Bedding also provides a comfortable resting surface and opportunities for behaviors such as digging, chewing, and nesting (Boyd,1988). Environmental conditions such as housing and husbandry have a major impact on the laboratory animal throughout its life and will thereby influence the outcome of animal experiments Baumans,(2004). Sanford et al. (2002) conducted experiment using different type of bedding material to study any external environment influence on mucosal immune system. Matsushita et al. (1975) stated that rats raised in wood shaving bedding showed decreased globulin levels than those kept on the other materials i.e. cotton wool, polypropylene fibers. In view of the above, the present investigation was therefore undertaken to study the potential impact of different bedding materials on immune response and serum biochemical profile of caged mice.

Materials and Methods

Conventionally reared fifty one Albino mice (supplied by Institute of Animal Health and Veterinary Biologicals, Kolkata) at three weeks of age were used in the experiment. All animals were stained with crystal violet initially and then individually marked on the tail by a colored waterproof marker at weekly interval. The enrichment used in this study was based on the outcome of several previous experiments about the preferences of mice for wood based bedding type Sanford (2002) and Weerd (1994).Three types of bedding viz. (a) chopped paddy straw (CPS) , (b) neem

wood shavings (NWS) and (c) paddy husk (PH) were steam sterilized at 130° C for 20 minutes and applied at a depth of 2 cm in cages and changed at weekly interval. For this study NWS bedding was considered as enrichment object. The mice were provided ad libitum acidified water (pH 2.8 to 3.1, monitored continuously) and provided with own formulated feed containing ingredients like crushed wheat, skimmed milk powder, cod liver oil, dried yeast, and sodium chloride (I.V.R.I) and that was autoclaved at 100°C for 58 min. Light intensity in the room was 200–260 lux, measured 1 m above the floor. A 12 h light (06:00–18:00) and 12 h (18:00-06:00) dark cycle was maintained throughout the study. Polypropylene solid floor cages 29 cm x 22 cm x 14 cm with a floor area of 638 cm2 (Tarson Lab wares) were used in the experiment. At the end of 16th week of age both male and female animals were euthanized by cervical dislocation. The heart, lungs, liver and spleen were removed and weighed. Intestine and spleen were utilized for immunological assays on the same day. Liver and heart were kept in - 40° c for estimation of reduced glutathione. The data was analyzed using one way ANOVA by SPSS (Ver. 10.00).

Immunological parameters

Isolation of intestinal mucosal leukocytes (IML): The IML were isolated from mice intestine with slight modification as adopted by (Grachia et al. 1997) which was the modified original method of (Lillehoj and Chung ,1992).

Isolation of spleenic lymphocytes : Spleens were aseptically resected and cell suspension was prepared by teasing the tissue in a sterile Petri dish using stainless steel blade (Grachia et al. 1997).

Superoxide anion production assay: Oxidative radical production by mucosal and blood leukocytes was assayed by the nitrobule tetrazolium (NBT) reduction test as described by (Siwicki et al. 1985).

In vitro Nitrate production assay: The production of reactive nitrogen intermediate (RNI) by spleenic and mucosal leucocytes was assessed by following the method described by Tafalla and Novoa (2000).

Lymphocyte proliferation assay: The test was carried out as described by (Plum et al. 1989).

Estimation of biochemical constituent

Estimation of total serum protein, Albumin and Globulin: Serum total protein and Albumin in each sample was determined by Biuret method (Reinhold, 1953) in a photoelectric colorimeter using an yellow green filter.

The globulin fraction in serum samples were calculated by subtraction of serum Albumin from total protein. The total serum protein, albumin and globulin were expressed as gm/dl of serum.

Estimation of Reduced Glutathione (GSH): Reduced glutathione was measured by the method described by (Griffith, 1980) in UV-VIS spectrophotometer at 412 nm wavelength.

Results and Discussion

Immunological parameters: To determine the external environmental influence on local and systemic immunity, intestinal mucosal leucocytes as well as blood and spleenic leukocytes were assayed respectively (Table1). The intestinal mucosa leukocytes were activated by NWS that lead to more functional and proliferative activity of leukocytes. Significant ($p<0.05$) production of reactive oxygen radical (0.71 ± 0.01) and reactive nitrogen radical (30.67 ± 0.88 µM) as well as higher S.I value (7.90 ± 0.17) for lymphocytes were observed in NWS in comparison to CPS and PH. Even though blood leukocytes showed increased reactive oxygen radical production, systemic macrophages and lymphocytes failed to respond against different bedding types. They undertook a study to observe the effect of hard wood based bedding and cotton bedding on intestinal mucosal immune response and concluded that B cell resident within Payers patches, Mesentric lymph nodules and lamina propria showed increased virus specific IgA production when mice were kept in hard wood bedding. In contrast, there was no difference in the viral IgG production. This result was in agreement with the work done by Sanford et al. (2002).

Enhanced immune response to hard wood in general, neem wood in specific, might be due to the presence of some immunomodulator component(s). One such immunomodulatory component might be saponin, that had been identified in plants (Sanford et al. 2002). Further, NB-II peptidoglycan (Biswas et al., 2002), Gallic acid (Chatterjee and Pakrashi, 1994), (–) epicatechin (Schmutterer,1995) and catechin (Singh et al.1996) might be the cause of immunomodulatory property in neem wood. NWS might cause the change of microflora population of murine small intestine and thus, affect mucosal immunity (Sanford et al. 2002).

Reduced glutathione: After euthanasia liver and heart were collected for estimation of reduced glutathione (GSH) and their mean values were presented in Table 2. The result represent that mean liver GSH for mice housed under NWS bedding showed significantly ($p<0.05$) higher values i.e. 8.54 ± 0.2 µM when compared to PH (7.09 ± 0.18 µM) and CPS bedding (6.96 ± 0.14 µM). It was observed that there was no significant ($p<0.05$) variation among heart GSH value between different bedding types (Table2).

This result was in accordance to the study conducted by (Potgieter et al. 1996). They reported significantly higher liver GSH value for rat housed in wood-derived bedding material (pine wood) than that of vermiculite and eucalyptus pulp beddings.

Table 1: Mean ± S.E of O.D value of Superoxide anion production assay, $NaNo_2$ (µM) of in vitro nitrite production assay and Stimulation Index (S.I) of lymphoproliferation assay for different bedding types

Bedding	O.D value		NaNo2 (µM)		S.I	
	IML	Blood	IML	SL	IML	SL
PH	0.23 ± 0.01b	0.31 ± 0.03b	17.0 ± 1.15b	18.67 ±1.86	6.33 ± 0.21 b	6.88 ± 0.29
NWS	0.71 ± 0.01a	0.41 ± 0.01a	30.67 ± 0.88a	22.0 ± 2.31	7.90 ± 0.17 a	6.69 ± 0.32
CPS	0.33 ± 0.03b	0.31 ± 0.02ab	15.67 ± 1.20b	19.0 ± 0.58	6.46 ± 0.27 b	6.47 ± 0.26

Mean values bearing common superscripts do not differ significantly. ($p<0.05$) ($n = 3$)

Table 2: Mean ± SE of reduced glutathione in liver and heart in different bedding types

Bedding	Liver (µmole/ gm of tissue)	Heart (µmole/ gm of tissue)
PH	7.09 ± 0.18b	5.3 ± 0.21
NWS	8.54 ± 0.2a	5.53 ± 0.26
CPS	6.96 ± 0.14b	5.26 ± 0.29

Mean values bearing common superscripts do not differ significantly. ($p<0.05$) ($n = 3$)

Enzyme Glutathione peroxidase (GPx) is involved together with GSH in the protection of organism against reactive oxygen species. GSH has many important functions in the cell. It directly participates in the scavenging of free radicals: hydrogen peroxide, superoxide anion and hydroxyl radicals. GPx catalyses the formation of oxidized glutathione (GSSG) from GSH during the reduction of free radicals (Parke, 1991). Variations in GSH level during oxidative stress may result from modification in synthesis. The mechanisms responsible for maintenance of GSH homeostasis in different tissues was poorly documented Hussain and Jakoniuk (2004).

Depleted level of reduced glutathione in animals caged in PH and CPS bedding might be due any unidentified substance emanated from these bedding materials caused GSH involvement in scavenging of H2O2. This reaction is catalysed by GPx. As a result of this reaction, GSH is oxidized and therefore the reduced glutathione level is diminished.

Serum Biochemical parameters: Mean serum TP, albumin and globulin are presented in Table 3. The present study gives inconsistent result, that serum total protein and albumin values were significantly ($p<0.05$) higher for animals caged in NWS, but globulin value was not statistically significant.

The result was not in accordance with (Matsushita et al. 1975), as the report states that there was decrease in globulin value when rats were housed with wood shaving bedding.

The possible reason for increased level of total protein, albumin and globulin in NWS bedding might be due to metabolic alteration brought about by the chemical constituents present in this bedding type.

References

1. Baumans, V. (2004). The welfare of laboratory mice. In: Kaliste E, ed. The welfare of Laboratory Animals. Dordrecht: Kluwer Academic Publishers,pp.119-152.
2. Biswas, K., Chattopadhyay, I., Banerjee, R.K. and Bandyopadhyay, U. (2002). Biological activities and medicinal properties of neem.(Azadirachta indica). *Current Science*.,82(11/10).
3. Boyd, J. (1988). Mice: Humane Innovations and Alternatives in Animal Experimentations., 3.98-99.
4. Chatterjee, A. and Pakrashi, S. (1994). The Treatise on Indian Medicinal Plants.3:76.
5. Grachia, F.J.S., Aller, W.W. and Mccormack, W.T. (1997). Impaired calcium mobilization and different tyrosine phosphorilation in intestinal intraepithelial

Table 3: Mean ± SE of serum total protein (TP), albumin and globulin for enriched and non-enriched bedding type with n=3

Bedding	TP (g/dl)	Albumin (g/dl)	Globulin (g/dl)
PH	5.77 ± 0.23b	3.89 ± 0.06b	1.88 ± 0.17
NWS	7.04 ± 0.04a	4.34 ± 0.06a	2.71 ± 0.1
CPS	6.01 ± 0.13b	4.07 ± 0.06b	3.26 ± 1.41

lymphocytes. *Immunology*,91:81-87.
6. Griffith, O.W.(1980). Determination of glutathione using reductase and 2-vinylpyridine. *Animal Biochem.* 150:76.
7. Hussain, L.A. and Jakoniuk,J.M. (2004). Liver catalase, glutathione reductase and peroxide activity in acute intoxication with Chlorfenvinphos, an organophosphate Insecticide. *Polish Journal of Environmental studies* 13(3):303-309.
8. Lane-Petter.(1972) The Laboratory mouse. The UFAW Handbook on the Care and management of Laboratory Animals (4th edition). Churchill Livingstone, Edinburgh,pp.187-203.
9. Lillehoj, H.S., and Chung, K.S.(1992). Postnatal development of T- lymphocytes subpopulations in the intestinal intraepithelium and lamina propria in chickens. *Vet. Immunol. Immunopathol.*,31:347.
10. Matsushita, H., Matsumoto, H., and Ando, H. (1975). Influence of polypropylene fiber on the growth and haematologic values in rats. Bull Azabu. *Vet. coll. 30:* 179-186.
11. Reinhold, J.G.(1953). Standards method of clinical chemistry , edited by M. Reiner, Academic press, New York and London 1, pp-88.
12. Parke, D.V.(1991). Nutritional requirements for detoxication of environmental chemicals. *Food. Add. Contam*.,8:381.
13. Plum, J.A., Milroy, R. and Kaye, S.B.(1989). Effect of pH dependence of 3-2-5 diphynyl tetrazolium bromide forzaman absorption on chemico sensitivity determined by a novel tetrazolium based assay. *Cancer Res.*,49:4435-4440.
14. Potgieter, F.J., Torronen, R., and Willke, P.I. (1996). The invitro effect of different bedding materials on the antioxidant levels of rat heart, lung, and liver tissue. *J.S. Afr. Vet. Assoc*.,67(1):27-30.
15. Sanford, A.N., Clark, S.E., Talham, G. and Coffin, S.E.(2002). Influence of bedding type on mucosal immune responses. *Comprarative Medicine*.,52(5): 429-432.
16. Schmutterer, H.(1995) The neem tree: Source of unique natural products for integrated pest management, *Medicine*.,52(5):429-432.
17. Singh, R.P., Chari, M.S., Raheja, A.K. and Kraus, W.(1996). Neem and environment. Oxford & IBH Publishing, New Delhi,i/ii:1-1198.
18. Siwicki, A.K., Klein,P. and Studnica, M.(1985). Immunostimulatory effect of dimerized lipozyme(KLP-602) on non specific defence mechanism and protein against furunculinosis. *Veterinary Immunology* 61:369-378.
19. Tafalla, C. and Novoa, B.(2000) Requirement of nitric oxide production by turbot head kidney macrophages. *Dev. Comp. Immunol*.,24:623-631.
20. Weerd, V.H.A., Baumans, V. and Colas, J.M.(1994). Strain specific behavioral responses to environmental enrichments in the mouse. *J. Exp. Anim. Sc*.,36:117-127.

* * * * * * * *