

## EVALUATION OF STRESS AND ITS EFFECTS ON THE IMMUNE SYSTEM OF HAND-REARED MULE DEER FAWNS (*Odocoileus hemionus*)<sup>□</sup>

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**Abstract:** High mortality often occurs when many mule deer fawns (*Odocoileus hemionus*) are hand-raised together. Thymic atrophy frequently was observed in those that died. No specific pathologic agents could be identified. It was thought that there may be an adrenal corticoid-induced immunosuppression due to the stress of hand-rearing many fawns together. To study this problem, fawns were taken from the doe at two days of age and divided into four groups of five each. Two groups were from tame does and two from recently trapped wild does. Twins were separated into a single feeder group and into a multiple feeder group. There were no differences in stress between the groups as determined by urinary cortisol and corticosterone/creatinine ratios. Humoral immunity following *Clostridium* toxoid vaccination was determined by immunodiffusion. Cell-mediated immunity was determined by dinitrochlorobenzene skin tests. Serum protein electrophoresis, WBC counts, and weight gain were monitored. All groups showed similar weight gains, and humoral and cell-mediated immune responses. Serum gamma globulins, and total serum proteins were higher and segmented neutrophils lower in the fawns from the wild does throughout the duration of the eight-week study. This would indicate that these fawns had a greater passive immunity as a result of the ingestion of more colostrum, which most likely occurred as a result of better maternal care by the wild does.

### INTRODUCTION

Tame deer are necessary and commonly used in studies of food habits, energetics, behavior, and other endeavors related to deer ecology.<sup>1,8</sup> These studies have created a demand for large numbers of tame deer. To obtain tame deer, fawns must be removed from their dams within 2 days of birth and be raised by hand so they will become imprinted on human handlers. Fawns have been obtained from two sources; tame does which were previously hand-reared, and wild does trapped during February and March and held in captivity until their fawns are born in June and July. Previous attempts at hand-rearing large numbers of these fawns (20 to 50)

over a 7-year period have yielded a 46% survival rate, with mortality being as high as 92%.<sup>1,2</sup> Losses in nearly all instances were due to diarrhea. Necropsies were routinely conducted. Although the clinical signs always were quite similar, no known pathogenic agents were found. The only consistent necropsy findings were a mild enteritis and small or nearly absent thymic glands.

It was thought that management and training programs were causing a continued stress to the pregnant does as well as the fawns. Automobile traffic, humans, strange animals and surroundings, all seemed to contribute to the problem, particularly for the wild does who were extremely excitable through-

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out their 6 to 8 months of captivity. The fawns appeared to be under the greatest stress during the first few weeks of life as a result of: 1) removal from their dam, 2) multiple feeders or surrogate mothers which appeared quite disturbing to them, 3) constant introduction of new fawns, and 4) frequent human activity in the area.

Stress stimulates increased secretion and high plasma levels of glucocorticoids.<sup>15</sup> Glucocorticoids have immunosuppressive properties that affect inflammatory, humoral, and cell-mediated immune responses. Cortisol acts to suppress the exudative phase of inflammation by inhibiting the movement of white blood cells and plasma proteins to the site where microorganisms and their products accumulate.<sup>6,8</sup> This inhibition prevents the development of the localized suppression of the infectious process. Cortisol is also responsible for stabilization of membranes, inhibition of lactic acid production, and inhibition of enzyme systems of leucocytes.<sup>2,16</sup> Negation of these factors reduces the bactericidal effect of the inflammatory response.

Humoral immunity is inhibited by glucocorticoid suppression of corticoid sensitive B and T lymphocytes.<sup>2,4,14</sup> Suppression of these cells in some cases leads to decreased antibody production.

The non-specific, and proliferation phases of the cell-mediated immune response are also altered by glucocorticoids.<sup>1,2,4,19</sup> The non-specific factors affected are the macrophage aggregation factor lymphokine, and the migration inhibitory factor lymphokine. Disturbance of both lymphokines may impair macrophage function and antigen processing. Cortisol suppression of lymphocyte proliferation may alter the intensity of the cell mediated immune response by suppressing T cells and by causing a lymphopenia.

Stress in the dams during pregnancy and in the young following birth inhibits

the development of the thymus or causes thymic atrophy.<sup>15</sup> Fetal thymectomy in sheep reduces delayed type hypersensitivity reactions, and there are fewer perivascular aggregations of mononuclear cells.<sup>10</sup> In addition there is a 30% reduction in circulating lymphocytes. Growth of the lymphoid apparatus and production of lymphocytes, however, continues to occur, and antibody production in both the primary and secondary immune responses are unaffected.<sup>10</sup>

These effects of stress on the immune system, the high mortality in the absence of a demonstrable or consistent pathogenic agent, the always present thymic atrophy as compared to wild fawns, and the prolonged stress on the dams during pregnancy and on the fawns during rearing indicates the possible presence of a stress-induced immunosuppression or deficiency as being a major factor responsible for the high mortality experienced in hand rearing deer fawns in the manner described. To examine this possibility humoral and cellular immunity, and factors associated with the immune system (white blood cell counts, protein electrophoresis, etc.) were studied and differences in stress evaluated in fawns raised by multiple feeders versus a single feeder and in fawns from wild does versus those from tame does.

## MATERIALS AND METHODS

Five wild does (*Odocoileus hemionus*) were trapped in February and transported in individual crates several hundred kilometers to the pens they would occupy until parturition. Five tame does were held in similar, adjacent pens. The pens, which were located two km from a residential area, were 50 by 100 m in size and contained a three-sided weather-tight shelter with dirt floors. Barriers from feeders and other humans were provided for the does to hide behind. Does were fed good quality alfalfa hay, a concentrate mix, and water *ad libitum*.

All fawns were born in June and were twins. Each fawn was allowed to stay with the dam for 48 h to encourage maximal colostrum intake. After removal, each was weighed, its navel disinfected with iodine, and the fawn was then vaccinated with *Clostridium perfringens* type C and D antitoxin. Twins were split into a single feeder and multiple feeder groups. The two groups were placed in identical and adjacent pens. They had access to an open-sided weather-tight shelter with dirt floors, dry bedding, heat lamps, and water and good alfalfa hay *ad libitum*. All were fed identical amounts of a standardized formula of 6 parts whole milk, 3 parts evaporated milk and 1 part buttermilk. One-half ml of a standard vitamin formula containing vitamin B complex and iron intended for infants was given twice a day for the first 3 weeks of life. The fawns in the single feeder group had minimal contact with other people, whereas those in the multiple feeder group were fed alternately by 8 different feeders and frequently were exposed to additional people.

Stress was evaluated by measuring cortisol, corticosterone and calcium/creatinine ratios in the urine.<sup>15</sup> Urine was collected once a week by manual tickling of the vulva or prepuce. Urine cortisol and corticosterone levels were determined by radioimmunoassay as described by Hasler *et al.*<sup>7</sup> Urine calcium was determined by standard atomic absorption spectrophotometric methods. Urine creatine levels were determined by the color reaction with alkaline picrate.<sup>16</sup>

Humoral immunity was evaluated by determining antibody production to a specific antigen vaccination. The fawns were vaccinated subcutaneously with 3 ml of *Clostridium* toxoid<sup>17</sup> at 4, 6, and 8

weeks of age. Serum collected at weekly intervals was analyzed by microimmunodiffusion, as described by Crowle,<sup>5</sup> for the detection of precipitins to these toxoids. The weekly serum samples were assayed with toxins<sup>17</sup> corresponding to all of the bacterial toxoids contained in the vaccine administered, except *Clostridium chauvoei*. The development of precipitation lines between the serum and toxin during the immunodiffusion technique indicates the presence in the serum of the antitoxin precipitins produced by the fawns in response to the toxoids in the vaccine. The fawns were considered to be capable of active humoral immunity when serum antitoxins were first detected against two of the toxins.

Cell-mediated immunologic responsiveness was assayed using contact sensitization to 1-nitro,2,4-dichlorobenzene (DNCB). The sensitizing properties of DNCB are related to its ability to act as a hapten forming covalent bonds with lysine groups of epidermal proteins.<sup>3</sup> DNCB was applied and skin biopsies were taken as described by Trindle *et al.*<sup>17</sup> to determine the intensity of the cellular response. Intensity was rated on a scale of 1 to 4 according to external lesions, skin thickening, histologic lesions and infiltration of lymphocytes.<sup>17</sup>

The fawns were weighed and jugular blood samples (5 ml) were taken weekly. Total and differential white blood cell counts and hematocrits were determined. The remainder of the blood sample was centrifuged and serum obtained for protein electrophoresis, immunodiffusion for antibody production, and the determination of total serum protein concentration by refractometer.

Urine and blood parameters, and body weight were analyzed by age of fawns in

<sup>15</sup> Developed by Hycel, Inc., Houston, Texas

<sup>16</sup> Jen-Sal Electroid 7, Jensen-Salabery Lab., Kansas City, Mo. (*Clostridium chauvoei*, *septicum*, *novyi*, *sordellii*, *perfringens* types C and D)

<sup>17</sup> Produced by Norden Labs, Lincoln, Nebraska

weekly intervals by three methods: 1) a one-way analysis of variance by treatment group, 2) a paired T test for twin fawns split into single and multiple feeder groups, and 3) an unpaired T test for fawns from wild or tame does. The least significant difference test was used as the mean separation test for the analysis of variance. All tests of significance were at  $\alpha$  0.05. When further reference is made concerning one of the above groups, the following abbreviations are used: s-w for single feeder-wild dam, s-t for single feeder-tame dam, m-w for multiple feeders-wild dam, m-t for multiple feeders-tame dam, S for all fawns in the single feeder groups, M for all fawns in the multiple feeder groups, W for all fawns born to wild does, and T for all fawns born to tame does.

## RESULTS

The rate of survival of the fawns from birth to weaning was 90%. Two of the 20 fawns in the experiment died within the first week of life. Both were from the m-t group. Thus, this group contained only three fawns while the other three groups each contained five. Necropsies of both fawns showed the presence of extremely small thymus glands with no known pathogen identified.

The collection of urine by genital tickling was successful for all fawns. Neither urine cortisol/creatinine ratios (Table 1) or urine corticosterone/creatinine ratios (Table 2) showed any significant differences between any of the groups at any age, with the exception of group M at 8 weeks of age in which urine corticosterone/creatinine ratios were significantly higher than group S.

Humoral immunity (Table 3) was recorded as the age in weeks of the first detectable antibody response to the challenge presented. All fawns, except one, were able to produce antibodies against the *Clostridium* vaccination between 6 and 8 weeks of age. Cell

mediated immunity (Table 4) of each fawn was graded on a scale of 1 to 4 with increasing intensity of reaction to the DNCB applied to the surface of the skin. All fawns demonstrated some degree of cellular immunity. There were no differences between any of the groups for either humoral or cellular immune responses.

Total serum protein (Table 5) was significantly higher for the first week for the s-w group as compared to the s-t group. In conjunction with this, the W group had significantly higher values than the T group for the first week. The higher total serum proteins were due to higher gamma globulin levels (Table 6).

White blood cell (WBC) counts for fawns from the tame does were higher than those from the wild does at 1 and 5 weeks of age. The differences in WBC counts were due to differences in the number of neutrophils (Tables 8 and 9).

Hematocrits were not different between any of the groups (Table 10). The fawns from the wild does were significantly heavier than those from the tame does, the third, fourth, and fifth weeks of age (Table 11).

## DISCUSSION

Even small changes in an animal's environment such as restraint and venapuncture can elevate plasma corticoid levels immediately and give a distorted indication of the presample stress confronting the animal. For this reason the collection of urine by genital tickling and the determination of corticoids was used as an indication of the degree of stress prior to collection. Creatinine ratios were used to remove the dilution affect created by different concentrations of urine. Although urinary creatinine excretion is increased by stress, the increase in comparison to that of glucocorticoids is negligible.<sup>15</sup> Urinary cortisol levels were greater and appeared to be a better indicator of stress than corticosterone, although cortisol

TABLE 1. Urine cortisol/creatinine ratios (pg/mg) in mule deer fawns.\*

Group	Age in Weeks							
	1	2	3	4	5	6	7	8
Single Feeder-Wild Dams	71.3**	129.6	1.7	3.1	3.4	34.1	36.7	1.6
	85.7	245.8	3.2	6.2	4.6	52.0	60.7	
	0.0	0.0	0.0	0.0	0.0	0.0	0.4	
	211.5	567.8	7.3	12.3	9.7	123.9	144.3	
Single Feeder-Tame Dams	260.2	233.8	47.7	68.3	12.0	12.3	34.3	57.4
	552.7	399.0	56.1	148.2	15.4	20.5	10.1	43.0
	2.0	0.0	0.0	0.0	0.0	0.0	27.1	26.9
	1298.6	944.5	123.5	333.3	37.9	35.9	41.4	87.8
Multiple Feeders-Wild Dams	110.0	185.6	170.7	130.6	19.0	29.5	110.9	4.5
	144.3	304.0	229.2	176.7	25.6	29.8	177.3	31.9
	24.9	3.8	5.0	0.0	1.0	0.0	8.9	22.4
	365.8	724.3	492.7	393.1	63.0	69.0	376.5	67.5
Multiple Feeders-Tame Dams	149.7	68.5	114.6	12.8	21.4	89.0	87.75	109.9
	215.8	104.7	126.9	7.1	26.0	152.8	129.1	154.9
	5.9	0.0	26.3	8.6	0.0	0.0	0.0	0.3
	397.8	189.0	260.0	21.0	50.4	265.5	175.5	219.4

\*n=5, except in the multiple feeders-tame dams group in which n=3.

\*\*Given in order:  $\bar{X} \pm SD$ , minimum, maximum.

TABLE 2. Urine corticosterone/creatinine ratios (pg/mg) in mule deer fawns.\*

Group	Age in Weeks							
	1	2	3	4	5	6	7	8
Single Feeder-	14.7**	51.7	30.0	26.8	22.2	15.5	8.8	0.0
Wild Dams	10.2	47.8	34.2	22.4	29.1	21.2	11.9	0.0
	5.1	0.0	1.2	0.1	0.0	0.1	0.0	29.2
	31.1	100.8	82.5	54.9	62.2	52.6		
Single Feeder-	39.2	12.5	8.3	10.7	3.9	11.6	9.8	13.5
Tame Dams	66.0	16.8	15.0	14.1	4.3	6.1	1.6	7.5
	2.7	0.0	0.0	0.0	0.0	5.6	8.6	8.2
	156.9	41.0	34.8	27.7	9.8	17.8	10.9	18.8
Multiple Feeders-	13.8	33.4	12.9	27.7	12.1	16.5	19.6	20.0
Wild Dams	8.6	20.1	9.6	36.3	14.7	11.5	21.2	12.3
	0.0	0.0	3.4	1.8	3.7	4.2	0.1	11.3
	23.4	52.3	25.1	88.9	38.2	27.3	44.1	28.7
Multiple Feeders-	15.7	27.9	45.9	5.6	8.6	16.8	10.3	30.0
Tame Dams	11.9	35.9	70.3	5.6	9.5	26.1	11.2	14.1
	2.0	4.3	2.2	0.0	1.4	0.5	2.4	20.0
	22.7	69.2	127.0	11.2	19.4	46.9	18.2	40.0

\*n=5, except in the multiple feeders-tame dams group in which n=3.

\*\*Given in order:  $\bar{X} \pm SD$ , minimum, maximum.

TABLE 3. Age of first immunoglobulin production to clostridium toxoid vaccination in mule deer fawns.

No. of Fawns	Age in Weeks	Fawns' Dam*
1	6	T
14	7	7T, 7W
2	8	W, W
1	0	W
2	Died	T, T

\*Fawns born to tame does (T) or wild does (W).

TABLE 4. Cell mediated immunity in mule deer fawns as indicated by delayed hypersensitivity response to topical application of DNCB.

No. of Fawns	Histological Grade*	Fawns' Dam**
1	+4	T
12	+3	5T, 7W
4	+2	T, T, W, W
1	+1	W
2	Died	T, T

\*+4 = greatest response, +1 = least response.<sup>16</sup>

\*\*Fawns born to tame does (T) or wild does (W).

TABLE 5. Total serum protein (g/100ml) in mule deer fawns.\*

Group	Age in Weeks							
	1	2	3	4	5	6	7	8
Single Feeder-Wild Dams	7.1**	7.0	6.8	6.6	6.3	6.2	6.7	6.8
Single Feeder-Tame Dams	6.4	6.9	6.4	6.3	6.2	6.2	6.6	6.7
Multiple Feeders-Wild Dams	6.9	6.8	6.7	6.3	6.4	6.0	6.6	6.6
Multiple Feeders-Tame Dams	6.6	6.9	6.6	6.6	6.2	6.2	6.6	6.8

\*n=5, except in the multiple feeders-tame dams group in which n=3.

\*\*Given in order:  $\bar{X} \pm SD$ , minimum, maximum.

TABLE 6. Plasma total gamma globulin concentrations (g/100ml) in mule deer fawns.\*

Group	Age in Weeks							
	1	2	3	4	5	6	7	8
Single	1.8**	1.4	1.0	0.7	0.7	0.6	0.6	0.7
Feeder-	0.6	0.4	0.4	0.2	0.1	0.2	0.1	0.1
Wild	1.1	1.0	0.6	0.5	0.5	0.4	0.5	0.5
Dams	2.3	1.8	1.6	1.0	0.8	0.8	0.7	0.8
Single	1.2	1.0	0.9	0.7	0.6	0.5	0.7	0.7
Feeder-	0.2	0.1	0.1	0.2	0.2	0.0	0.2	0.1
Tame	1.0	0.8	0.7	0.6	0.5	0.4	0.5	0.6
Dams	1.4	1.1	1.0	1.0	0.9	0.5	1.0	0.9
Multiple	1.7	1.2	0.9	0.7	0.7	0.5	0.6	0.6
Feeders-	0.5	0.3	0.3	0.3	0.5	0.2	0.1	0.2
Wild	0.9	0.9	0.5	0.3	0.3	0.3	0.4	0.4
Dams	2.1	1.6	1.4	1.2	1.5	0.8	0.7	0.8
Multiple	1.5	1.1	0.8	1.1	0.7	0.7	0.7	0.8
Feeders-	0.2	0.4	0.4	0.1	0.3	0.3	0.3	0.3
Tame	1.3	0.7	0.4	1.0	0.4	0.5	0.5	0.5
Dams	1.7	1.4	1.1	1.2	1.0	1.1	1.0	1.0

\*n=5, except in the multiple feeders-tame dams group in which n=3.

\*\*Given in order:  $\bar{X} \pm$  SD, minimum, maximum.TABLE 7. Total leukocyte counts (cells  $\times 10^3/\text{mm}^3$ ) in mule deer fawns.\*

Group	Age in Weeks							
	1	2	3	4	5	6	7	8
Single	3.7**	6.4	4.7	4.4	3.8	4.0	3.7	4.1
Feeder-	1.5	5.4	1.3	1.6	0.5	1.3	0.6	0.9
Wild	1.4	3.3	2.9	2.1	3.2	2.9	3.0	3.0
Dams	5.0	15.2	6.4	6.5	4.3	6.2	4.1	5.4
Single	6.5	6.6	5.7	5.4	5.0	6.5	6.1	5.5
Feeder-	1.0	3.8	1.3	2.0	1.3	4.6	3.3	1.6
Tame	5.4	3.5	4.0	3.4	3.4	2.0	2.0	3.6
Dams	7.5	13.0	7.7	8.0	6.8	11.9	9.8	7.7
Multiple	4.2	4.5	4.9	4.7	4.0	4.0	5.0	3.8
Feeders-	1.4	1.4	1.2	1.6	1.3	1.3	1.0	0.7
Wild	2.2	3.3	3.3	3.3	2.8	2.4	4.2	3.0
Dams	5.9	6.1	6.5	6.8	5.5	5.3	6.4	4.5
Multiple	7.6	11.1	5.8	7.8	5.0	4.8	5.0	5.7
Feeders-	1.4	2.5	1.9	4.2	1.0	0.7	2.0	0.7
Tame	6.0	8.5	4.7	4.8	3.9	4.2	3.8	5.1
Dams	8.6	13.5	8.0	10.8	5.9	5.5	7.3	6.5

\*n=5, except in the multiple feeders-tame dams group in which n=3.

\*\*Given in order:  $\bar{X} \pm$  SD, minimum, maximum.



TABLE 8. Lymphocyte counts (cells  $\times 10^3/\text{mm}^3$ ) in mule deer fawns.\*

Group	Age in Weeks							
	1	2	3	4	5	6	7	8
Single	1.5**	2.0	1.8	1.9	1.8	1.5	1.6	1.6
Feeder-	0.8	1.3	0.7	1.3	0.3	0.4	0.4	0.4
Wild	0.9	0.9	0.9	0.9	1.6	0.9	1.2	1.0
Dams	2.4	3.8	2.7	4.2	2.2	2.0	2.0	2.0
Single	1.4	1.6	1.6	2.1	1.7	1.5	2.2	1.7
Feeder-	0.1	0.9	0.2	0.3	0.4	0.4	0.7	0.3
Tame	1.3	0.8	1.2	1.7	1.1	1.0	1.4	1.4
Dams	1.5	2.9	1.8	2.4	2.2	2.1	3.0	2.2
Multiple	1.2	1.1	1.6	2.0	1.4	1.4	2.0	1.4
Feeders-	0.3	0.3	0.7	0.8	0.3	0.4	0.6	0.3
Wild	1.0	0.7	0.9	1.0	1.0	0.9	1.3	1.1
Dams	1.6	1.3	2.7	3.2	1.7	1.8	2.8	1.8
Multiple	0.9	1.8	2.2	2.5	1.5	1.5	1.9	1.6
Feeders-	0.05	0.8	1.1	1.2	0.1	0.4	0.4	0.04
Tame	0.9	0.9	2.2	2.6	1.3	1.2	1.4	1.6
Dams	1.0	2.6	3.4	3.3	1.6	2.0	2.2	1.6

\*n=5, except in the multiple feeders-tame dams group in which n=3.

\*\*Given in order:  $\bar{X} \pm \text{SD}$ , minimum, maximum.TABLE 9. Neutrophil counts (cells  $\times 10^3/\text{mm}^3$ ) in mule deer fawns.\*

Group	Age in Weeks							
	1	2	3	4	5	6	7	8
Single	2.1**	4.8	2.5	2.2	1.8	1.9	1.9	2.3
Feeder-	0.4	3.8	1.0	1.9	0.4	0.4	0.4	0.8
Wild	1.7	1.5	1.7	0.3	1.4	1.4	1.6	1.3
Dams	2.4	10.2	4.3	5.2	2.3	2.3	2.3	3.5
Single	5.2	4.5	3.7	2.9	3.0	4.7	3.7	3.2
Feeder-	0.1	2.6	1.1	1.7	0.9	4.0	2.5	1.3
Tame	5.3	2.0	2.7	1.1	2.1	0.8	0.6	1.5
Dams	6.1	8.7	5.6	5.2	4.4	9.6	6.4	4.4
Multiple	3.0	3.1	3.1	2.3	2.4	2.4	2.6	2.0
Feeders-	1.2	1.1	1.6	0.9	1.2	0.9	0.6	0.8
Wild	1.5	2.4	1.4	1.5	1.2	1.3	1.7	1.1
Dams	4.5	4.4	5.0	3.5	3.9	3.1	3.0	3.0
Multiple	6.2	8.8	3.2	5.0	3.1	3.1	2.8	3.6
Feeders-	1.2	2.5	1.1	5.4	0.9	0.9	1.7	1.0
Tame	5.0	6.0	2.2	1.2	2.1	2.5	1.5	2.6
Dams	7.5	10.4	4.4	8.9	3.9	4.1	4.7	4.6

\*n=5, except in the multiple feeders-tame dams group in which n=3.

\*\*Given in order:  $\bar{X} \pm \text{SD}$ , minimum, maximum.

Table 10. Hematocrits (%) in mule deer fawns.\*

Group	Age in Weeks							
	1	2	3	4	5	6	7	8
Single Feeder-	33**	30	33	33	31	31	32	39
Wild	2	6	2	3	6	2	3	2
Dams	31	20	31	29	23	29	28	39
Single Feeder-	35	35	36	38	38	35	36	42
Tame	30	32	31	31	31	32	34	37
Dams	7	1	1	1	2	3	4	4
Multiple Feeders-	19	31	29	30	29	28	28	32
Wild	36	33	32	33	34	35	39	44
Dams	31	29	30	30	31	29	35	37
Multiple Feeders-	5	4	4	3	2	3	4	4
Wild	24	25	25	27	29	26	31	31
Dams	36	33	35	34	35	32	40	41
Multiple Feeders-	32	31	31	32	31	33	36	38
Tame	5	1	2	2	3	3	1	2
Dams	29	30	28	30	27	30	35	36
Multiple Feeders-	37	32	32	33	33	36	36	39
Tame								
Dams								

\*n=5, except in multiple feeders-tame dams group in which n=3.

\*\*Given in order:  $\bar{X} \pm$  SD, minimum, maximum.

TABLE 11. Body weight (lbs) of mule deer fawns.\*

Group	Age in Weeks							
	1	2	3	4	5	6	7	8
Single Feeder-	8.2**	9.5	11.0	13.2	15.7	18.2	20.2	21.7
Wild	1.1	1.0	1.0	.9	1.2	1.0	1.4	2.3
Dams	7.2	8.5	10.0	12.2	13.7	16.7	18.0	18.2
Single Feeder-	9.5	10.5	12.2	14.5	16.7	19.2	21.5	23.5
Tame	8.4	9.7	11.7	13.4	15.6	17.4	18.8	19.4
Dams	0.7	0.9	0.9	1.4	1.6	1.4	1.3	0.4
Multiple Feeders-	7.7	8.7	10.7	12.2	13.5	15.0	17.5	19.5
Wild	9.5	11.2	13.0	15.0	17.2	18.7	20.2	20.2
Dams	7.5	8.4	9.6	11.5	13.5	16.0	17.8	20.7
Multiple Feeders-	0.6	1.4	1.7	2.0	2.5	3.1	3.1	4.0
Wild	6.5	6.0	6.7	8.0	9.2	10.7	13.0	14.2
Dams	8.2	9.2	11.2	13.0	15.7	18.7	21.2	24.0
Multiple Feeders-	8.3	9.8	10.9	12.5	14.1	15.7	18.5	19.3
Tame	0.8	0.4	0.6	0.7	1.0	1.6	3.3	3.8
Dams	7.7	9.5	10.2	13.0	13.2	14.5	16.5	16.0
Multiple Feeders-	9.2	10.2	11.2	13.2	15.2	17.5	22.2	23.5
Tame								
Dams								

\*n=5, except in multiple feeders-tame dams group in which n=3.

\*\*Given in order:  $\bar{X} \pm$  SD, minimum, maximum.

levels were more variable than those for corticosterone (Tables 1 and 2). Urine calcium excretion is known to increase in response to stress.<sup>15</sup> Because of the cost and limited availability for the determination of free glucocorticoids, urine calcium levels were measured and compared to those for cortisol. There was no correlation, however, and in this study urine calcium/creatinine ratios appeared to be a poor indication of stress.

There was a non-significant trend for higher cortisol/creatinine ratios in the fawns raised by multiple feeders than those raised by a single feeder. This correlated with the observation of increased stress in the fawns when a new feeder was introduced. The new feeder's presence caused unacceptance of the milk offered, withdrawal to the far end of the pen, and general excitement among the fawns. This occurred even though an individual familiar to them was present. The two fawns that died in the multiple feeder group showed similar responses.

The lower cortisol/creatinine ratios from the fawns in the single feeder group and visual observations indicates the acceptance of a known reliable milk source or surrogate mother. These fawns appeared unafraid at all feedings and never missed a feeding. They gained weight faster the first 5 weeks of age, although the 7th and 8th weeks of life the multiple feeder fawns' body weight began to approach that of those in the single feeder group (Table 8). Although not statistically significant, the correlation between lower cortisol/creatinine ratios and weight gain suggests that the single feeder provides an environment during feeding more conducive to the performance of the fawns than those raised by many feeders.

The amounts of stress present in this study did not measurably affect the fawns' immune system. Both humoral and cellular immune responses were observed. There were no differences in response between any of the groups (Table 3 and 4). In conjunction with this,

when comparing the multiple feeder groups with the single feeder groups no differences are observed in gamma globulin levels, or WBC counts of either lymphocytes or neutrophils. Stress induced glucocorticoid immunosuppression has been well demonstrated; although, high levels of exogenous steroids are necessary.<sup>1,4,7</sup> Levels of this magnitude were not demonstrated in this study and therefore do not appear to be involved in causing the extremely small thymus glands consistently present in fawns that died. It is still possible however, that smaller increases in glucocorticoid secretion associated with the increased stress of multiple feeder rearing of deer fawns may decrease their resistance to disease. Challenge with pathogenic agents may be necessary to determine if this occurs.

In the past it was thought that a higher mortality occurred in fawns born to wild, trapped does than in those born to tame does. This assumption, however, was based only on impressions and not on actual studies. This study does not support this assumption. As indicated by significantly higher plasma gamma globulin levels at one week of age (Table 10), the fawn born to wild does received more colostral immunoglobulins from their dams prior to their separation 2 days after birth. The wild does appeared to be better mothers than the tame does; therefore, it was thought that the higher plasma gamma globulin levels in their fawns occurred as a result of the ingestion of more colostrum. The higher plasma gamma globulin levels resulted in significantly increased total serum protein concentrations (Table 5). The amount of colostral immunoglobulins absorbed is known to be the single most important factor for disease prevention in a number of animal species.<sup>9,11,13</sup> Thus, the lower gamma globulin levels present in the fawns from tame does may have been responsible for their significantly higher WBC counts (Table 7), which were due to increased numbers

TABLE 12. Urine and blood parameters, and body weight for mule deer fawns.

Parameter	Age in Weeks							
	1	2	3	4	5	6	7	8
Urine Cortisol/	147.6*	163.9	80.2	61.5	13.7	39.5	67.0	60.8
Creatinine	299.7	279.4	140.5	126.4	18.9	71.3	108.0	77.2
Ratios	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.4
(pg/mg)	1248.6	944.5	492.7	393.1	63.0	265.5	376.5	219.4
Urine	21.4	31.8	21.9	18.6	11.4	15.2	12.5	18.1
Corticosterone/	34.8	32.8	33.6	23.9	16.6	16.2	13.9	13.3
Creatinine	0.0	0.0	0.0	0.0	0.0	0.1	0.0	0.0
Ratios (pg/mg)	156.9	100.8	127.0	88.9	62.2	52.6	44.1	40.0
Total	6.8	6.9	6.7	6.5	6.3	6.2	6.6	6.7
Serum	0.4	0.4	0.3	0.4	0.3	0.3	0.4	0.3
Protein	6.2	6.4	6.0	5.7	5.6	5.6	5.9	6.2
(g/100 ml)	7.5	7.6	7.2	7.2	6.8	6.7	7.5	7.1
Total Serum	1.5	1.2	0.9	0.8	0.7	0.6	0.7	0.7
Gamma	0.5	0.3	0.3	0.2	0.3	0.2	0.2	0.2
Globulin	0.9	0.7	0.4	0.3	0.3	0.3	0.4	0.4
(g/100 ml)	2.3	1.8	1.6	1.2	1.5	1.1	1.0	1.0
Total	5.1	7.3	5.2	5.2	4.4	4.8	5.0	4.7
Leukocyte	2.0	4.0	1.3	2.1	1.2	2.6	2.1	1.3
Counts	1.4	3.3	2.9	2.1	2.8	2.0	2.0	3.0
(cells × 10 <sup>3</sup> /mm <sup>3</sup> )	8.6	15.2	8.0	10.8	6.8	11.9	9.8	7.7

TABLE 12. (continued)

Lymphocyte Counts (cells $\times 10^3/\text{mm}^3$ )	1.3	1.6	1.8	2.0	1.6	1.5	2.0	1.6	1.6	2.0	1.6
	0.4	0.9	0.7	0.9	0.3	0.4	0.5	0.3	0.3	0.5	0.3
	0.9	0.7	0.9	0.9	1.0	0.9	1.2	1.0	1.0	1.2	1.0
	2.4	3.8	3.4	4.2	2.2	2.1	3.0	2.2	2.2	3.0	2.2
Neutrophil Counts (cells $\times 10^3/\text{mm}^3$ )	4.0	5.2	3.1	2.8	2.6	3.1	2.8	2.6	2.6	2.8	2.7
	1.9	3.2	1.2	2.1	0.5	2.4	1.6	0.5	0.5	1.6	1.1
	1.5	1.5	1.4	0.3	1.2	0.9	0.6	1.2	1.2	0.6	1.1
	2.5	10.4	5.6	8.9	4.4	9.6	6.4	4.4	4.4	6.4	4.6
Body Weight (lbs)	8.1	9.3	10.8	12.6	14.8	16.7	18.8	20.4	14.8	18.8	20.4
	0.9	1.1	1.3	1.5	1.9	2.1	2.2	3.0	1.9	2.2	3.0
	6.5	6.0	6.75	8.0	9.25	10.75	13.0	14.25	9.25	13.0	14.25
	9.5	11.25	13.00	15.0	17.25	19.25	22.25	24.00	17.25	22.25	24.00
Hematocrits (%)	31	30	31	31	31	31	34	38	31	31	38
	5	4	3	3	3	3	3	3	3	3	3
	19	20	25	27	23	26	28	31	23	26	31
	37	35	36	38	38	36	39	44	38	39	44

\* $\bar{X} \pm \text{SD}$ , minimum, maximum. (n=18).

of neutrophils (Table 8 and 9). This would indicate that the tame fawns, due to lower passive immunity, had greater infectious challenge which necessitated an increase in their white blood cells to combat it. We conclude that the wild does are as good or better than hand-raised

tame does as a source of fawns for hand rearing.

To assist further studies in which mule deer fawns are to be used, the urine and blood parameters as well as body weight have been summarized for all of the fawns used in this study (Table 12).

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