

**PRE-WEANING DIET AND STALL WEANING METHOD
INFLUENCES ON STRESS RESPONSE IN FOALS**

by

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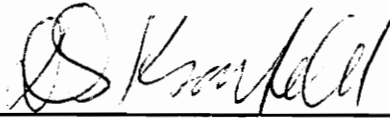
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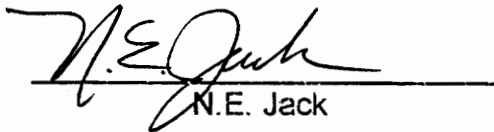
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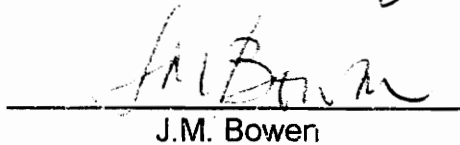
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ABSTRACT

The response of foals to the stress of weaning was examined in terms of a behavioral protocol and the responses of plasma ascorbate, serum cortisol, and the serum cortisol response to an ACTH challenge. Behavior scores (1 to 10) as an index of stress were assigned to each foal daily, with high scores indicating less stress and better adjustment. The experimental plan was a 2 X 2 factorial of pre-weaning diet and stall weaning method. Foals were raised on pasture supplemented with hay and a pelleted concentrate (PHC) or pasture supplemented with hay only (PH). Foals were placed in stalls singly or in pairs for weaning. Gender influences were also examined. The foals exhibited characteristic behavioral and physiological responses to the social dislocative stress of weaning. Behavior scores were lower in paired than in single foals ($p = .008$) and tended to be lower in PH than PHC foals ($p = .15$). No differences in post-weaning plasma ascorbate concentrations were found among treatments. Responses of serum cortisol to an ACTH challenge were lower in PH than PHC foals ($p = .001$) and in paired than single foals ($p = .058$), and lower responses were taken to represent adrenal depletion arising from stress. Behavior scores were positively correlated with the response of serum cortisol to ACTH. Both behavioral data and the serum cortisol response to ACTH indicate that foals were better able to cope with weaning stress when supplemented with concentrate prior to weaning and when weaned singly.

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INTRODUCTION

Stress physiology and behavior are important concepts in the evaluation of animal welfare (Fraser et al., 1975; Ewbank, 1985; Moberg, 1985). A central feature of stress is the release of adrenocorticotrophic hormone (ACTH) from the anterior pituitary, which is the primary regulator of cortisol secretion by the adrenal glands. This central concept has remained a useful starting point despite numerous modifications (Mason, 1975; Moberg, 1985). Changes in plasma cortisol concentrations have been shown to be a sensitive index of adrenocortical response (James et al., 1970). The pituitary-adrenal response is stimulated typically by emotional perturbations such as uncertainty or social dislocation (Dantzer and Mormède, 1985; Hart, 1985).

The stress of social dislocation is exemplified when foals are weaned. Traditional methods of weaning management abruptly separate mare and foal. For practical purposes, foals are often placed in stalls at weaning so they can be kept under close observation, easily caught and handled. It is a common belief that a stall companion eases the emotional stress of weaning, and to that end, foals are often weaned and stalled in pairs (Evans et al., 1990).

Previous weaning studies have examined behavioral and physiological responses in foals weaned using various separation protocols. Foals weaned by gradual as opposed to abrupt separation methods exhibited fewer behavioral signs of stress (McCall et al., 1985). Post-weaning plasma cortisol concentrations were elevated in both mares and foals (Malinowski et al., 1990).

Blood cortisol concentrations in most mammals are influenced by gender differences, cortisol concentrations being generally higher in female animals than in males (Critchlow et al., 1963; Gray, 1971).

Another feature of stress is the increased demand for ascorbic acid (Newberne and Conner, 1984). Blood ascorbate is depleted in horses with severe infections, long bone fractures and over-training (Jaeschke and Keller, 1978; Jaeschke, 1984). These findings suggest that blood ascorbate concentrations may serve as an indicator of stress in horses.

Previous studies in our laboratory have shown that foals exhibit characteristic behavioral and physiological responses to the social dislocative stress of weaning (J.L. Holland and K.M. Griewe-Crandell, unpublished data). The objective of this experiment was to evaluate and compare the effects of pre-weaning feeding management, stall weaning methods and gender of the animal on stress in weanling foals using a behavioral protocol, the responses of plasma ascorbate and serum cortisol, and the response of serum cortisol to an ACTH challenge.

REVIEW OF LITERATURE

Animal Welfare and Stress

Animal welfare may be studied in terms of stress physiology and behavior (Moberg, 1985; Kronfeld et al., 1989). Animal well-being is not universally defined, partly because people tend to judge the quality of animal life based on personal experiences. Most would agree that if an animal is experiencing prolonged or severe stress, its well-being is threatened. Thus measures of stress are relevant to animal welfare.

Animals maintain a dynamic equilibrium of molecular, cellular, physiological and behavioral conditions known as homeostasis (Cannon, 1932). Extra demands on homeostasis lead to a general adaptation syndrome or stress (Selye, 1976; Chrousos, 1992). Successful adaptation is achieved with a set of responses, physiological and behavioral changes that counteract stressors and usually help to re-establish homeostasis.

The original concept of stress has been modified in several ways to fit current research (Fraser et al., 1975; Moberg, 1985). For the purposes of this paper, stress will be addressed in three levels: eustress, mild distress and severe distress. (Selye, 1976; Ewbank, 1985; Kronfeld, 1989). *Eustress*, or "good stress," is harmless, expressed by mild behavior and physiological changes that may be beneficial for adaptation; *mild distress* covers the adaptive response expressed with some behavioral and physiological changes that may be indications of harm to the animal; *severe distress* depicts the damaging response expressed by undesired behavior and extreme physiological changes.

Stressor was the general term coined by Selye (1976) used to refer to various stimuli that threatened homeostasis. In the history of stress literature, stressors have included both physical (e.g., cold, fatigue, injury) as well as emotional (e.g., uncertainty, social dislocation) stimuli (Mason, 1975).

Distressed animals exhibit poor performance, and prolonged stress will result in economic loss. Exposure to social stress in chickens and swine reduced body weight gain, feed efficiency and resistance to infection (Gross and Siegel, 1981; Larson et al., 1985). It is usually beneficial for both the welfare of the animal and the profit of the manager to better understand the stress response and its effects.

Physiological Mechanisms

Short-term effects of stress are reflected in the autonomic nervous system, which provides the basis of the fight-or-flight response (Cannon, 1932). Hypothalamic neurons that generate corticotrophin-releasing hormone (CRH) also stimulate the central autonomic-arousal system, leading to catecholamine (epinephrine, norepinephrine) secretion from the adrenal medulla and certain autonomic nerves (Chrousos, 1992). Catecholamines have an immediate effect on heart rate, blood pressure, respiratory rate and metabolism, enabling the animal to make rapid physical adjustments to acute stress.

Corticotrophin-releasing hormone also initiates a coordinated series of behavioral responses, including improved alertness and attention span level, decreased reflex time and suppression of feeding and sexual behavior (Chrousos, 1992).

Longer-term effects of stress are reflected in the neuroendocrine system, which reacts slowly relative to the autonomic response. A key feature in this neuroendocrine system is the hypothalamic-pituitary-adrenal axis (Figure 1). Hypothalamic CRH neurons stimulate the anterior pituitary to secrete adrenocorticotrophic hormone (ACTH), which stimulates the adrenal cortex to secrete corticosteroids (Mol and Rijnberk, 1989). In the horse, cortisol is the major corticosteroid produced (James et al., 1970). Cortisol stimulates gluconeogenesis, decreases glucose utilization, mobilizes fatty acids and amino acids, stabilizes lysosomes, suppresses inflammation and the immune response (Guyton, 1991; Chrousos, 1992). This peripheral adaptation works to counteract the stressors and relieve stress. Negative feedback of cortisol directly affects the hypothalamus and the anterior pituitary, decreasing formation of CRH and ACTH. In the absence of stressful stimuli, ACTH is released in a frequent, pulsatile manner, regulated by endogenous corticosteroids. In the horse, blood cortisol concentrations have a diurnal variation with peak values at 0600 and lowest values at 1800 (Larrison et al., 1979).

Stressors encountered by the animal may reduce its immune resistance sufficiently to allow viral and secondary bacterial infections to occur (Roth, 1985). In general, the number of blood lymphocytes decreases and the number of heterophils increases in response to stressors. The ratio of heterophil to lymphocyte has been used as an effective indicator of stress in chickens, and it has been recommended for assessment of long-term environmental changes, as opposed to blood corticosteroid concentration, which is a better measure of short-term changes. (Gross and Siegel, 1983). Glucocorticoids reduce the number of circulating lymphocytes, monocytes and eosinophils (Osbaldiston and

Johnson, 1972; Wilder, 1992). Glucocorticoids suppress the immune response at all levels, affecting lymphocyte proliferation and differentiation, inhibiting natural killer cells and antibody-forming B cells, and they affect the development of different T cell subpopulations (Roth, 1985; Wilder, 1992).

Stress is characterized by hypermetabolism, which causes tissue protein mobilization and nitrogen loss, increased fatty acid oxidation and increased oxygen consumption (Wilmore, 1977; Donoghue, 1989). The stress response is associated with increased urinary excretion of nitrogen, phosphorus, vitamins A, E and C, iron, zinc and creatine (Scrimshaw, 1977; Stoner, 1987; Hensle and Askanazi, 1988).

Blood concentrations of phosphorus, zinc, iron and manganese decrease in response to stress; in contrast, copper concentrations in blood increase (Orr, 1990; Chirase et al., 1991; Madsen et al., 1991). Increased blood copper concentration is attributed to the stimulation of ceruloplasmin synthesis and release from the liver; decreased blood zinc concentration is thought to be secondary to liver metallothionein synthesis and subsequent sequestering of zinc in the liver (Keen and Graham, 1989; Bremner and Beattie, 1990). It was implicated recently that the mineral requirements of cattle, especially those of zinc, copper and manganese, change in response to stress (Chirase, 1994).

ACTH Response Test

Stimulation of the adrenal cortex by an intramuscular injection of ACTH gel causes a rapid rise in plasma cortisol concentration in the horse that peaks between 5 and 8 h (James et al., 1970). The ACTH response test examines the function of the adrenal using administration of a known quantity of exogenous

ACTH (Piomb, 1991), and it has been useful in the diagnosis of pituitary tumors (Beech, 1987). The critical feature of the test is that the existence of adrenal reserve is proven by a substantial increase in blood cortisol concentrations in response to exogenous ACTH (Rijnberk and Mol, 1989).

Interpretation of the ACTH response test is not unequivocal (Moberg, 1985). In general, animals experiencing beneficial eustress have relatively high concentrations of serum cortisol prior to the ACTH challenge and a large rise in serum cortisol in response to the challenge, reflecting hypertrophy and increased secretory function of the adrenal cortex. Animals experiencing mild or moderate distress have higher concentrations of serum cortisol prior to the ACTH challenge and a less marked response to the challenge, reflecting partial depletion of the adrenal cortex by endogenous ACTH. Animals experiencing severe distress may have either high or low concentrations of serum cortisol prior to the ACTH challenge and a poor response to the challenge, reflecting exhaustion of the adrenal cortex and resultant inability to secrete more cortisol. This interpretation is illustrated in Figure 2.

Ascorbic Acid

Ascorbic acid is a known antioxidant in several reactions, acting as a cofactor for various hydroxylases, one of which is involved in the synthesis of adrenocortical steroids; another is essential for catecholamine synthesis (Newsholme and Leech, 1984). In sled dogs, plasma ascorbate declined during the racing season and was correlated with performance ratings; dietary supplementation of ascorbic acid offset the decline (Donoghue et al., 1987).

Ascorbic acid is depleted in chickens experiencing environmental stress; dietary supplementation of ascorbic acid enhanced resistance to disease (Gross, 1988).

Horses are capable of maintaining sufficient blood ascorbic acid concentrations under normal conditions (Stillions et al., 1971), but the need for ascorbic acid increases during stress (Newberne and Conner, 1984). Blood ascorbate is depleted in horses with severe infections, long bone fractures and over-training (Jaeschke and Kellier, 1978; Jaeschke, 1984). These findings suggest that blood ascorbate concentrations may serve as an indicator of stress in horses. Dietary supplementation of ascorbic acid has been an effective method of increasing blood ascorbate concentrations in horses according to some reports (Snow et al., 1987) but not in others (Pearson et al., 1943; Löscher et al., 1984).

Previous Weaning Studies in Foals

Traditional methods of weaning management abruptly separate mare and foal. McCall et al. (1985) examined abrupt or partial separation of mare and foal, finding that foals abruptly separated spent more time walking and trotting and vocalized more frequently. The study also indicated that in abrupt separation management, less activity was exhibited during the experimental period in foals having access to pre-weaning creep feed than in foals that had no pre-weaning creep feed.

In a companion paper, McCall et al. (1987) examined the physiological responses of foals weaned by abrupt or gradual methods, using multiple ACTH response tests and measuring basal and peak plasma cortisol concentrations, plasma concentrations of triiodothyronine (T3) and thyroxine (T4), weight gains

and feed consumption. It is questionable if multiple ACTH response tests are an effective measure of stress, since the response test is designed to evaluate adrenal function using a maximal challenge. Adrenal responses to ACTH challenges administered in succession may be affected by prior challenges. McCall et al. (1987) interpreted their findings to indicate that larger physiological responses to stress were exhibited by foals weaned by abrupt separation management than foals weaned gradually.

Another practice in weaning management is to place the foal in a stall at weaning time so that it can be kept under close observation, easily caught and handled. It is a common belief that a stall companion eases the emotional stress of weaning, and to that end, foals are often weaned and stalled in pairs (Evans et al., 1990). Malinowski et al. (1990) evaluated the influence of single or paired weaning on stress levels in foals using plasma cortisol concentrations and lymphocyte proliferation response. They noted increased plasma cortisol concentrations post-weaning in both mares and foals, but they did not find differences between single or paired weaning treatments. However, they suggested that the traditional management practice of paired weaning was more stressful than weaning singly, noting that foals weaned in pairs exhibited a decreased cell-mediated immune response.

Previous weaning studies have not reported correlations between behavior and physiological variables. Ewbank (1985) suggests that to establish a relationship between behavior and stress, evidence must meet four criteria: 1) stressors must be quantified; 2) physiological responses must be quantified and correlated with behavioral changes; 3) behavioral changes must be quantified, and 4) well-being must be damaged. . Few papers satisfy these criteria.

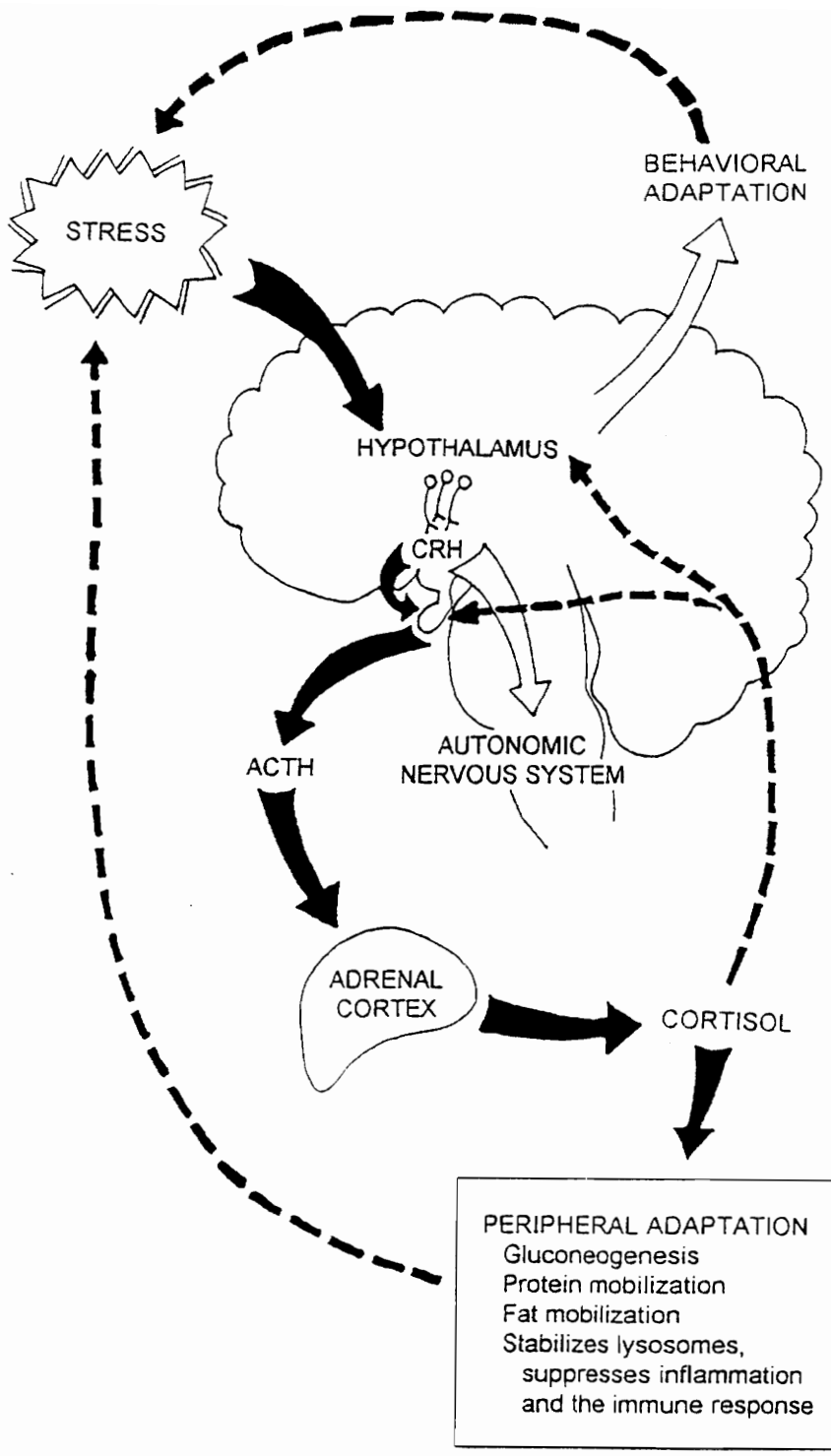


Figure 1. The neuroendocrine hypothalamic-pituitary-adrenal response (Munck et al., 1984; Moberg, 1985; Chrousos, 1992). Solid arrows and open arrows indicate stimulatory action; broken arrows indicate inhibitory action. Stress stimulates the hypothalamus, and hypothalamic corticotropin-releasing hormone (CRH) neurons stimulate the anterior pituitary to secrete adrenocorticotropic hormone (ACTH), which stimulates the adrenal cortex to secrete cortisol. Cortisol stimulates a peripheral adaptation that works to counteract the stressors and relieve stress. Negative feedback of cortisol affects the hypothalamus and the anterior pituitary to decrease production of CRH and ACTH.

Table 1. An interpretation of the ACTH response, formulated from various sources (Beech, 1987; Kronfeld, 1989; Rijnberk and Mol, 1989).

| Level of Stress | Serum Cortisol Concentration | |
|------------------------|--|---|
| | Pre-ACTH | Post-ACTH |
| "Normal, non-stressed" | relatively low basal concentrations | marked response, (increased concentrations) |
| Eustress | elevated concentrations | very large response |
| Mild Distress | high concentrations as compared to normal or eustressed | decreased response, probably smaller than normal or eustressed response |
| Severe Distress | either very high or very low concentrations as compared to other stress levels | little or no response |

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ABSTRACT

The stress response of foals during weaning was examined in terms of a behavioral protocol and the responses of plasma ascorbate, serum cortisol and the serum cortisol response to an ACTH challenge. The experimental plan was a 2 X 2 factorial of two pre-weaning diets and two stall weaning methods. Dietary groups included foals raised on pasture supplemented with hay and a pelleted concentrate (PHC) and foals raised on pasture supplemented with hay only (PH). Stall weaning methods included foals placed in stalls singly or in pairs. Gender influences were also examined. The foals exhibited characteristic behavioral and physiological responses to weaning stress. Behavior scores indicated fewer outward signs of stress in single than paired foals ($p = .008$) and tended to indicate less signs of stress in PHC than PH foals ($p = .15$). No differences in plasma ascorbate concentrations were found among treatments. Responses of serum cortisol to an ACTH challenge were lower in PH than PHC foals ($p = .001$) and in paired than single foals ($p = .058$), and lower responses represent adrenal depletion arising from stress. Behavior scores were positively correlated with the response of serum cortisol to ACTH. Both behavioral data and the ACTH response indicated that foals may cope better with weaning when supplemented with concentrate prior to weaning or when placed singly in stalls rather than in pairs.

Key Words: stress, weaning, ACTH, behavior

Introduction

Stress physiology and behavior are important concepts in the evaluation of animal welfare (Fraser et al., 1975; Ewbank, 1985; Moberg, 1985). A central feature of stress is the release of adrenocorticotrophic hormone (ACTH) from the anterior pituitary, which is the primary regulator of cortisol secretion by the adrenal glands. This central concept has remained a useful starting point despite numerous modifications (Mason, 1975; Moberg, 1985). Changes in plasma cortisol concentrations have been shown to be a sensitive index of adrenocortical response (James et al., 1970). The pituitary-adrenal response is stimulated typically by emotional perturbations such as uncertainty or social dislocation (Dantzer and Mormède, 1985; Hart, 1985).

The stress of social dislocation is exemplified when foals are weaned. Traditional methods of weaning management abruptly separate mare and foal. For practical purposes, foals are often placed in stalls at weaning so they can be kept under close observation, easily caught and handled. It is a common belief that a stall companion eases the emotional stress of weaning, and to that end, foals are often weaned and stalled in pairs (Evans et al., 1990).

Previous weaning studies have examined behavioral and physiological responses in foals weaned using various separation protocols. Foals weaned by gradual as opposed to abrupt separation methods exhibited fewer behavioral signs of stress (McCall et al., 1985). Post-weaning plasma cortisol concentrations were elevated in both mares and foals (Malinowski et al, 1990).

Blood cortisol concentrations in most mammals are influenced by gender, cortisol concentrations being generally higher in female animals than in males (Critchlow et al., 1963; Gray, 1971).

Another feature of stress is the increased demand for ascorbic acid (Newberne and Conner, 1984). Blood ascorbate is depleted in horses with severe infections, long bone fractures and over-training (Jaeschke and Keller, 1978; Jaeschke, 1984). These findings suggest that blood ascorbate concentrations may serve as an indicator of stress in horses.

The objective of this experiment was to evaluate and compare the effects of pre-weaning feeding management, stall weaning methods and gender of the animal on stress in weanling foals using a behavioral protocol, plasma ascorbate, serum cortisol and the response of serum cortisol to an ACTH challenge.

Materials and Methods

The experiment was conducted at the Virginia Tech MARE Center in 1993. The plan was a 2 X 2 factorial with eighteen Thoroughbred foals, two diets and two weaning methods. The mares were randomly divided prior to the birth of the foals into two groups: the PHC group was fed a diet consisting of pasture supplemented with hay and a pelleted concentrate formulated to meet or exceed NRC requirements; the PH group was fed pasture supplemented with hay only. The nutrient profiles of samples taken from the pastures, hay and concentrate, as analyzed by the Virginia Tech Forage Testing Lab, are summarized in Table 2. The animals were allowed *ad libitum* access to a trace mineral salt (Table 3). Estimated daily intakes of trace elements provided by the PH and PHC diets and the trace mineral salt, assuming likely intakes, are shown in Table 4.

Each group had nine foals; there were four PHC fillies and two PH fillies. Anthelmintic, vaccination and hoof trimming schedules routine to the Virginia Tech MARE Center were followed (Ley et al., 1992). Age at weaning was 5 to 7 mo.

The weaning study was conducted over a two week period, with each group being evaluated for a 4 d experimental period. The foals in each group were separated from their dams all at once and placed into stalls. The mares were abruptly removed from sight and earshot of the foals. Within each dietary group, four foals were weaned as two pairs, and five foals were weaned singly. Regardless of treatment, each foal was within sight of another foal. Stalls with

14 m² of available space were used to house the single foals. The paired foals were housed in larger stalls having 19 m² of available space.

During the experimental period, the foals were handled minimally for data collection purposes only, and the barn was treated as a closed environment with restricted access to visitors. The foals were bedded on straw and given *ad libitum* access to mixed grass hay and water. No pelleted concentrate was fed to either group during the experimental period. The stalls were cleaned and bedded at the same time each morning.

Daily ethograms were constructed for each foal over a 30 min observation period, generally between 1500 and 1700, with four or five foals being observed concurrently (Table 5). Previous experiments conducted at the Virginia Tech MARE Center associated characteristic behavioral and physical responses of foals with the social dislocative stress of weaning (J.L. Holland and K.M. Griewe-Crandell, unpublished data). The responses are summarized in a scheme using Selye's concepts of eustress and distress (Selye, 1976) and modeled after levels of stress in farm animals and sled dogs (Ewbank, 1985; Kronfeld et al., 1989). Behavior quantified included vocalizations (whinnies), frequency and duration of locomotor activity, eating, and responses attributed to stress such as weaving, pawing, rearing or wood chewing. Vocalizations were recorded as frequencies per 30 min, while other behavioral data were recorded as cumulative intervals of time spent in the activity. Locomotor activity was primarily divided into time standing, walking or laying. Standing time was subdivided into time when calm or disquieted (e.g., pawing, head weaving), and walking time was subdivided into time spent walking calmly or nervously. Stance

transitions (e.g., transitions from standing to walking) were recorded as frequencies per 30 min.

A behavioral score was assigned to each foal using the rating scale proposed by Kronfeld et al. (1989). A foal that exhibited three or more signs of severe distress (Table 5) was assigned a behavioral score of 1. One or two signs of severe distress justified a score of 2 or 3, and foals that exhibited signs of mild distress were assigned a score of 4, 5 or 6. Foals exhibiting signs of eustress were assigned a score of 7, 8 or 9. The score of 10 was reserved for foals that did not exhibit any signs of agitation and remained calm and alert.

Jugular blood samples were taken at 24 and 48 h post-weaning for plasma ascorbate analysis by HPLC (Shiiep et al., 1987). Blood was collected in heparinized tubes, immediately placed in the dark on ice and centrifuged within 30 min of collection. An aliquot of plasma was acidified with 5% metaphosphoric acid with a ratio of 1:4 plasma:acid (Shiiep et al., 1987). The acidified plasma was stored frozen in the dark pending completion of the ascorbate analysis.

An ACTH response test was conducted at 48 h post weaning. A blood sample was taken from each foal prior to an ACTH challenge of 1 IU/kg BW repository corticotrophin injection (H.P. Acthar Gel, Rorer Pharmaceuticals, Fort Washington, PA) injected IM (Plumb, 1991). A second blood sample was taken 5 h later. Serum cortisol concentrations were analyzed in both samples using a radio immunoassay procedure (Coat-A-Count Cortisol, Diagnostic Products, Los Angeles, CA). The baseline samples for the ACTH response test were taken between 1000 and 1100, and the post-ACTH challenge samples were taken

between 1500 and 1600 in order to minimize effects of diurnal variation (Larsson et al., 1979).

Data on blood variables were subjected to analysis of variance using GLM procedures (SAS, 1990), with diet, weaning method and gender in the model. Behavior variables were analyzed using GLM procedures of SAS (1990) with repeated measures over time, with diet, weaning method and gender in the model. Correlations of behavioral and physiological data were examined using multiple regression procedures (Ott, 1988; SAS, 1990). Nutrient means of the pasture, hay and concentrate samples were compared using a Student's *t* test.

Results and Discussion

Influences of separation on behavior

The effects of pre-weaning dietary treatment, stall weaning method and gender of the foal on behavior are shown in Figures 2 and 3. PHC foals, compared to PH foals, vocalized less ($p = .028$), tended to stand less ($p = .117$), and had a higher frequency of stance transitions ($p = .006$). They also tended to spend more time eating ($p = .243$) and have higher behavior scores ($p = .153$). These behavioral differences indicate a better adaptation to weaning by PHC than PH foals.

Foals weaned singly, compared to paired foals, spent more time standing ($p = .066$) and had higher behavior scores ($p = .008$). No care-soliciting or care-giving behavior was shown among the paired weanlings; they exhibited agonistic behavior toward each other, generally by flattening their ears, biting or threatening to kick. These observations suggested that pairing foals at weaning had little or no apparent advantage.

Colts, compared to fillies, spent more time eating ($p = .082$) but showed no other behavioral differences.

All behaviors changed with time after weaning as shown in Figure 4. Behavioral data recorded between 72 and 96 h post-weaning are not shown because there were no differences in behavior between 48 and 72 h and 72 and 96 h. At 24 to 48 h, compared to 0 to 24 h, foals vocalized less ($p = .010$) and spent more time standing ($p = .017$). At 48 to 72 h, compared to 24 to 48 h, foals vocalized less ($p = .093$) and spent more time laying ($p = .011$). At 48 to 72 h, compared to 0 to 24 h, foals vocalized less ($p = .0001$), spent more time

standing ($p = .075$), more time laying ($p = .006$), and more time eating ($p = .076$). Fewer behavioral signs of stress were exhibited as time post-weaning increased, as shown in the overall behavior scores (Figure 4), indicating that foals became adjusted over time.

Plasma Ascorbate

Plasma ascorbate concentrations of the foals were not affected by pre-weaning diet, weaning method or gender at 24 or 48 h (Figure 5). Plasma ascorbate concentrations were relatively low in the weaned foals compared to values reported for normal horses (Table 6). This data suggests that plasma ascorbate may be depleted at weaning; additional data are needed on this point. Horses have the capacity to maintain sufficient blood ascorbic acid concentrations under normal conditions (Stillions et al., 1971), but the demand for ascorbic acid is increased during stress (Newberne and Conner, 1984). Blood ascorbate was depleted in horses with severe infections, long bone fractures and over-training (Jaeschke and Keller, 1978, Jaeschke, 1984). In sled dogs, plasma ascorbate declined during the racing season and was correlated with performance ratings; dietary supplementation of ascorbic acid offset the decline (Donoghue et al., 1987). Dietary supplementation of ascorbic acid has been an effective method of increasing blood ascorbate concentrations in horses according to some reports (Snow et al., 1987) but not others (Pearson et al., 1943; Löscher et al., 1984). The possible advantages of ascorbic acid supplementation at weaning should be evaluated.

Serum Cortisol

Mean serum cortisol concentrations corresponding with pre-weaning dietary treatment, stall weaning method and gender of the foal are shown in Figure 6. Pre-ACTH serum cortisol concentrations were higher in PH weanlings than in PHC weanlings ($p = .014$), indicating that PH foals were stressed to a greater degree. Pre-ACTH serum cortisol concentrations tended to be higher in paired foals than in single foals ($p = .130$), indicating that paired foals may have adjusted less well to weaning stress. Malinowski et al. (1990) determined that post-weaning plasma cortisol concentrations are elevated in both mares and foals, but they did not find differences in plasma cortisol concentrations due to weaning foals singly or in pairs.

Plasma ascorbate concentrations at 24 h post-weaning correlated negatively with pre-ACTH serum cortisol concentrations ($p = .028$). Low plasma ascorbate would be associated with high plasma cortisol during mild and moderate stress, though plasma cortisol may decline during severe distress when the adrenal gland is exhausted (Kronfeld et al., 1989).

ACTH Response

The responses of serum cortisol to an ACTH challenge corresponding with pre-weaning dietary treatment, stall weaning method and gender of the foal are shown in Figure 6. The increase in serum cortisol concentrations following the ACTH challenge was lower in PH weanlings than in PHC weanlings ($p = .001$). We interpret the higher initial serum cortisol values in the PH weanlings, compared to the PHC weanlings, to indicate more stress; lower cortisol responses to ACTH in PH than PHC weanlings indicate less adrenal reserve.

Indications reflected by behavior and pre-ACTH serum cortisol concentrations of PHC and PH weanlings further support this interpretation. Thus, foals fed concentrate prior to weaning were better able to cope with weaning stress.

The response of serum cortisol to the ACTH challenge was lower in paired than in single foals ($p = .058$). These data support the indication reflected by pre-ACTH serum cortisol concentrations.

Serum cortisol concentrations before ($p = .0001$) and after ($p = .003$) the ACTH challenge were higher in fillies than colts, however, there was no difference in response of serum cortisol to ACTH challenge between fillies and colts; thus the ACTH response did not show that one sex was better able to cope with weaning stress than the other. Serum cortisol in most mammals is influenced by gender; cortisol concentrations are generally higher in females than in males. (Critchlow et al., 1963, Gray, 1971).

The ACTH response test was conducted at 48 h post-weaning in order to evaluate the degree of stress that the foals were experiencing. Interpretation of the ACTH response test is not unequivocal (Moberg, 1985). In general, animals experiencing beneficial eustress have relatively high concentrations of serum cortisol prior to the ACTH challenge and a large rise in serum cortisol in response to the challenge, reflecting hypertrophy and increased secretory function of the adrenal cortex. Animals experiencing mild or moderate distress have higher concentrations of serum cortisol prior to the ACTH challenge and a less marked response to the challenge, reflecting partial depletion of the adrenal cortex by endogenous ACTH. Animals experiencing severe distress have either high or low concentrations of serum cortisol prior to the ACTH challenge and a

poor response to the challenge, reflecting exhaustion of the adrenal cortex and resultant inability to secrete more cortisol.

Using this interpretation, our findings indicate that the physiological response to stress was less intense in PHC than PH foals and in single than paired foals. McCall et al. (1987) found that foals had a lower plasma cortisol response to exogenous ACTH when weaned by gradual as opposed to abrupt separation.

The behavioral data was examined for correlation with physiological variables. Plasma ascorbate concentrations 24 h post-weaning correlated negatively with eating time ($p = .041$) and negatively with total standing time ($p = .106$). Pre-ACTH challenge cortisol concentrations were negatively correlated with eating time ($p = .063$). The change in serum cortisol concentrations correlated positively with time spent walking calmly ($p = .033$). Eating time was positively correlated with time spent standing calmly ($p = .012$) and walking calmly ($p = .001$). In contrast, frequency of vocalization correlated positively with nervous walking ($p = .030$). All vocalizations performed were classified as whinnies which have been considered a distress or long-range communication call (Feist and McCullough, 1976). A significant negative correlation between basal plasma ACTH levels and locomotor activity has been observed in pigs (Mormède et al., 1984). Brain (1972) notes the relation of behavioral and physiological responses, indicating that ACTH suppresses locomotor activity and thereby influences other behaviors dependent upon locomotor activity.

The behavior scores were positively correlated ($p = .005$) with the rise of serum cortisol in response to exogenous ACTH (Figure 7). This correlation adds

importance to each result because it illustrates a relationship between behavioral observations and physiological responses.

Weanlings having lower behavior scores exhibited more outward signs of distress, and this distress was reflected in a lower ACTH response, indicating less adrenal reserve. Likewise, weanlings having higher behavior scores exhibited fewer outward signs of distress, and their physiological response indicated more adrenal reserve, or less adrenal depletion due to stress. The positive correlation of ACTH response and behavior score reinforces the acceptance of both assessments as valid indicators of weaning stress.

No ACTH response values for foals were found in the literature, and it would be of benefit to have “normal” values representing the “non-stressed” foal for future reference. In this study, the ACTH response test was used post-weaning after the stress was present. Although pre-weaning response tests would have provided “normal” values for the foals on this study, the maximal challenge on the adrenal prior to weaning would have affected post-weaning ACTH response results, thus biasing the experiment.

Influences of Pre-Weaning Diet on Stress Response

The pasture and hay had ample energy and protein. The nutrient profiles of the forages and concentrates consumed by the foals in this study (Table 2) are different in mineral content. In the concentrate, as compared to pasture, there were greater quantities of phosphorus ($p = .000002$), zinc ($p = .000005$) and copper ($p = .000003$). Iron quantities were higher in the concentrate as compared to the hay ($p = .002$).

The concentrations of phosphorus and zinc in the pasture and hay were lower than the recommended minimum requirements of .34% and 40 ppm, respectively, and the concentrations of copper in the pasture and hay marginally satisfied the 10 ppm minimum requirement (NRC, 1989). The intake of the trace mineral salt provided may be reasonably assumed at 30 mg/d and would have provided intakes of trace minerals much less than those provided by the feeds (Table 4).

The advantage of feeding concentrate may have been partly due to its mineral content. Stress, such as trauma and infection, increases requirements for phosphorus, iron and zinc (Wilmore, 1977). Shipping stress may change requirements for copper, zinc and manganese in calves (Chirase, 1994). In the present study, iron and manganese provided by the forages were well above the minimum requirements. On the other hand, the forages provided phosphorus, zinc and copper below minimum requirements (NRC, 1989), so their supplementation may have contributed to the amelioration of stress associated with feeding the concentrate. Future investigation of nutrition for foals before weaning should include an assessment of mineral requirements. A diet enriched for weaning stress could be advantageous in future horse production systems.

Implications

Stress is complex, and discrete variables such as certain behaviors, plasma ascorbate and serum cortisol provide only a partial indication of the complete picture. The combination of behavior scores and the ACTH response test indicated that provision of concentrate prior to weaning may positively influence a foal's ability to cope with weaning stress. Pairing foals at weaning had no apparent advantage.

Table 2. Nutrient profiles (mean \pm SD on a dry matter basis) of forages and pelleted concentrate consumed.

| | Pasture (n = 20) | Hay (n = 12) | Concentrate (n = 3) |
|---------|---------------------|-----------------|------------------------|
| CP, % | 18.5 \pm 3.6 | 14.9 \pm 3.1 | 17.8 \pm 1.7 |
| ADF, % | 30.1 \pm 3.3 | 36.1 \pm 5.1 | 13.3 \pm 3.7 |
| TDN, % | 67.8 \pm 3.7 | 61.2 \pm 4.0 | 79.6 \pm 2.9 |
| Ca, % | .45 \pm .12 | .62 \pm .25 | .76 \pm .08 |
| P, % | .30 \pm .04 | .26 \pm .04 | .73 \pm .04 |
| Mg, % | .19 \pm .03 | .23 \pm .07 | .33 \pm .04 |
| K, % | 1.25 \pm .44 | 1.07 \pm .35 | .74 \pm .33 |
| Na, ppm | 87.0 \pm 77.6 | 87.4 \pm 29.8 | 1915 \pm 251 |
| S, ppm | 2245 \pm 809 | 2045 \pm 425 | 2058 \pm 130 |
| Zn, ppm | 25 \pm 4.1 | 22 \pm 4.0 | 186 \pm 7.6 |
| Cu, ppm | 10.0 \pm 2.4 | 8.1 \pm .88 | 47.0 \pm 10.4 |
| Mn, ppm | 66.0 \pm 19.5 | 51.2 \pm 15.3 | 218 \pm 33 |
| Fe, ppm | 197 \pm 168 | 93.3 \pm 44.5 | 312 \pm 30.7 |

Table 3. Mineral profile of trace mineral salt supplemented.

| Mineral | Concentration, ppm |
|-----------|--------------------|
| Iron | 50 |
| Manganese | 20 |
| Zinc | 20 |
| Copper | 4 |
| Iodine | .2 |
| Cobalt | .07 |

Table 4. Daily intakes of trace elements provided by PH and PHC diets, and trace mineral salt (TMS), assuming likely intakes.

| Daily intake | TMS 30 g | PH 9.0 kg | PHC 7.35 kg PH 1.0 kg concentrate |
|--------------|-------------|--------------|---|
| Zn, mg/d | .6 | 225 | 370 |
| Cu, mg/d | .12 | 90 | 121 |
| Mn, mg/d | .6 | 594 | 703 |
| Fe, mg/d | 1.5 | 1773 | 1760 |

Table 5. Typical behavioral and physical signs of stress observed in stalled weanling foals.

| Eustress | Mild Distress | Severe Distress |
|------------------------------------|--------------------------|-------------------------|
| Whinnies occasionally | Whinnies frequently | Silent |
| Alert, attentive | Inattentive, distracted | Depressed, unresponsive |
| Stands well | Paws, weaves, chews wood | Stands head lowered |
| Walks calmly | Walks nervously or trots | Immobile |
| Seeks company (et-epileptic) | Avoids company | Ignores company |
| Epileptic (gives care & attention) | Agonistic (aggressive) | Apathetic |
| Eats well | Eats poorly | Refuses to eat |

Table 6. Blood ascorbic acid concentrations (mean \pm SE) reported for normal horses.

| Source | Reported value, $\mu\text{g/mL}$ |
|---------------------------|----------------------------------|
| Jaeschke and Keller, 1978 | 5.9 ± 1.4 |
| Stillions et al., 1971 | $3.9 \pm .3$ |
| This study | |
| 24 h post-weaning | $2.14 \pm .24$ |
| 48 h post-weaning | $1.95 \pm .23$ |

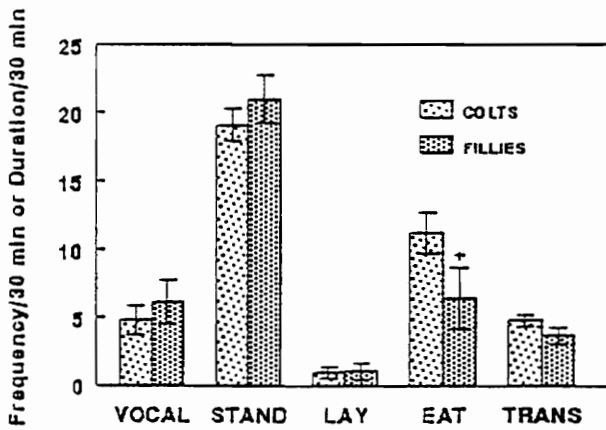
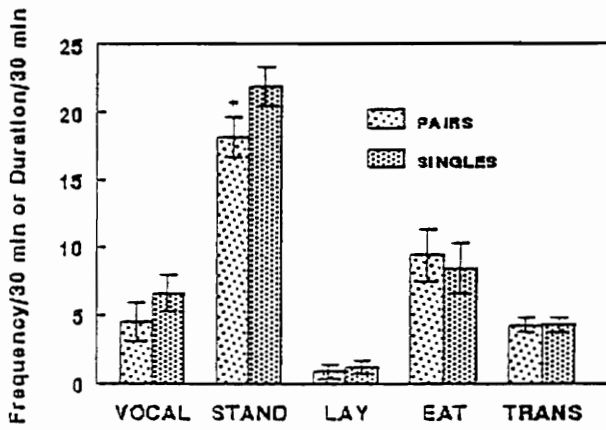
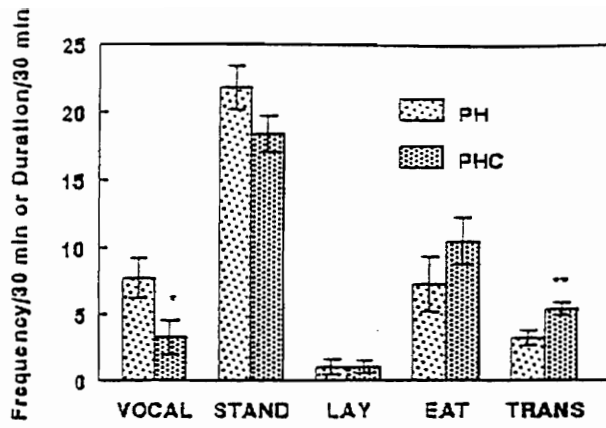


Figure 2. Influences of diet (upper), weaning method (middle), and gender (lower) on frequencies of vocalization and stance transitions per 30 min, duration of time spent standing, laying and eating per 30 min. Symbols indicate p values as follows; + ($p < .10$), * ($p < .05$), ** ($p < .01$), *** ($p < .001$).

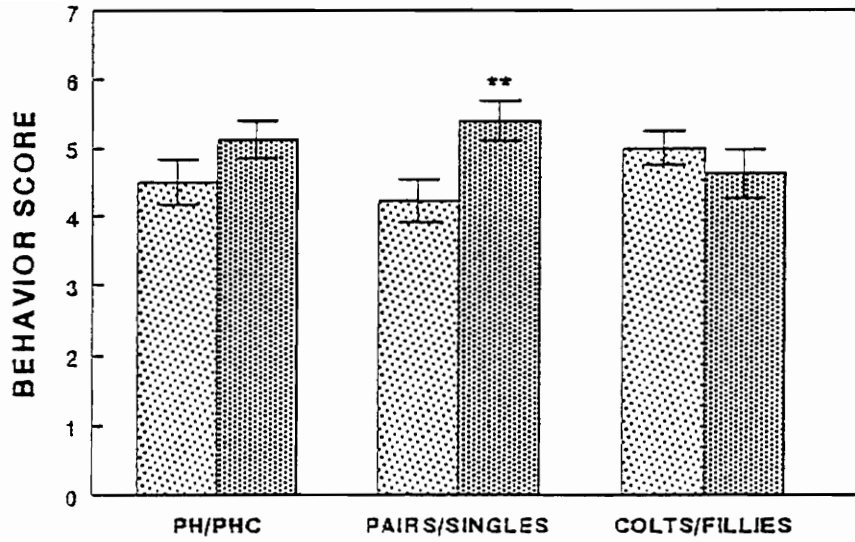


Figure 3. Influences of diet (left), weaning method (middle), and gender (right) on behavior scores of weaned foals. Note that higher behavior scores indicate lower stress. ** indicates $p < .01$.

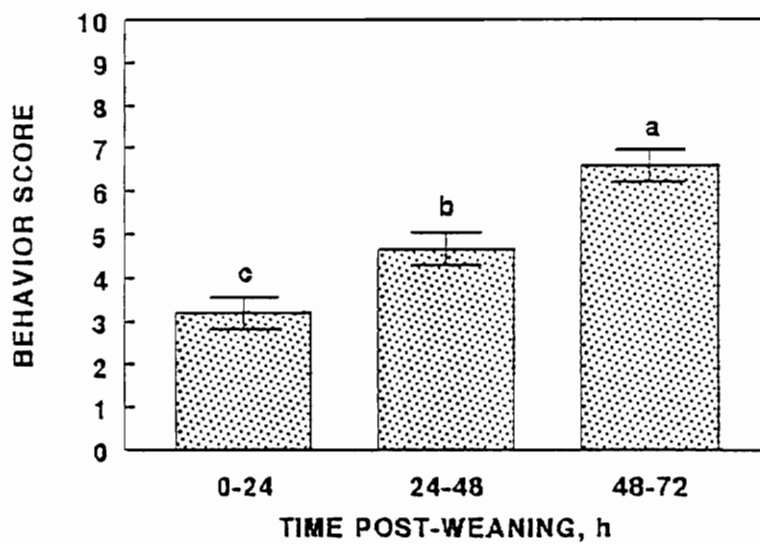
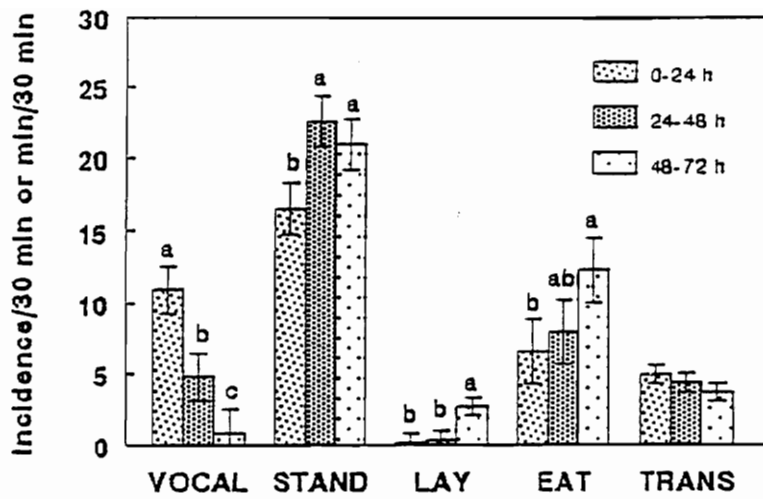


Figure 4. Changes in frequency of vocalizations and stance transitions (trans) per 30 min and duration of time spent standing, laying and eating per 30 min in foals over time post-weaning (upper). Changes in overall behavior score with time post-weaning (lower). Note that higher behavior scores indicate lower stress. Differing letters indicate $p < .10$ (upper) and $p < .01$ (lower).

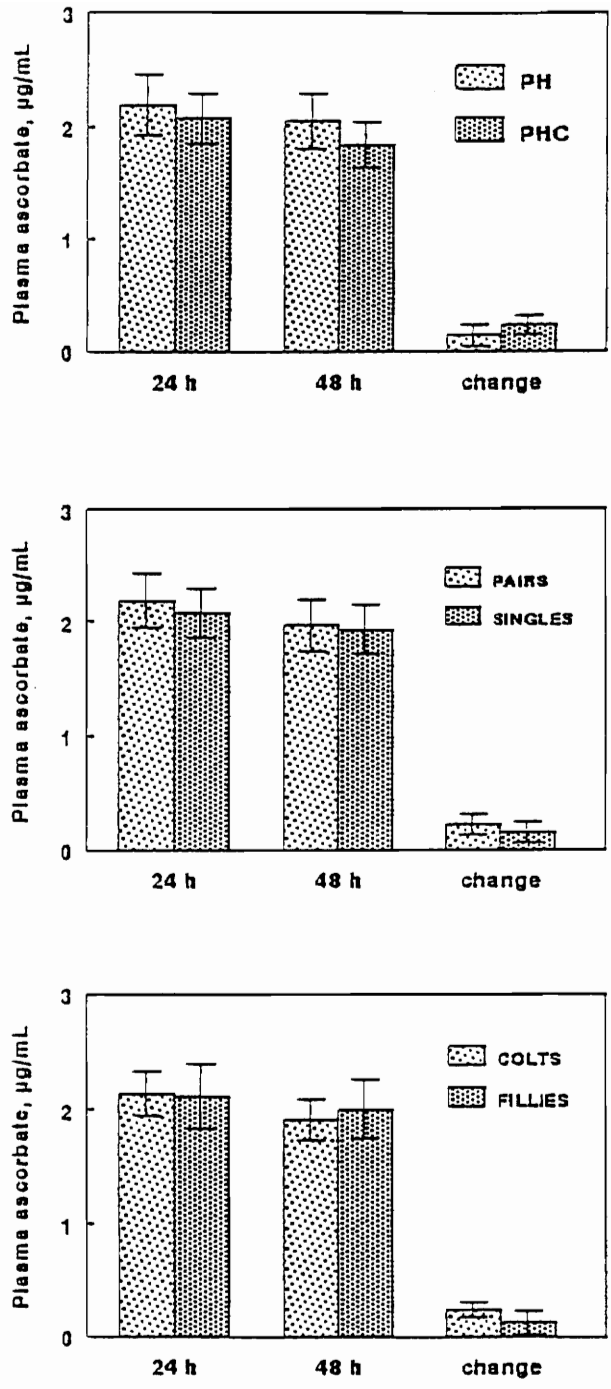


Figure 5. Influences of diet (upper), weaning method (middle), and gender (lower) on plasma ascorbate concentrations at 24 and 48 h post-weaning.

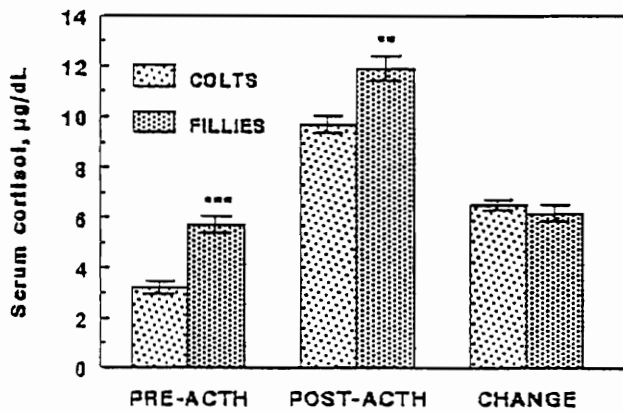
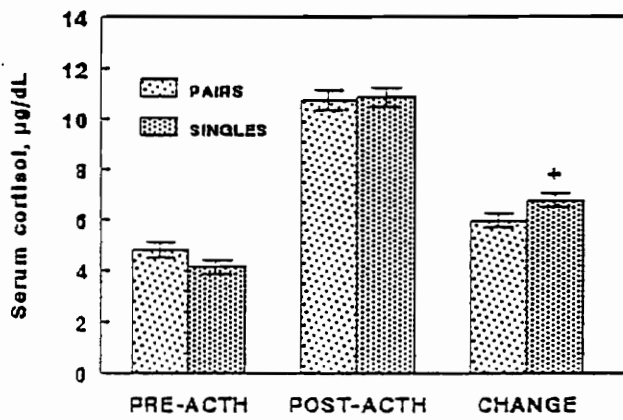
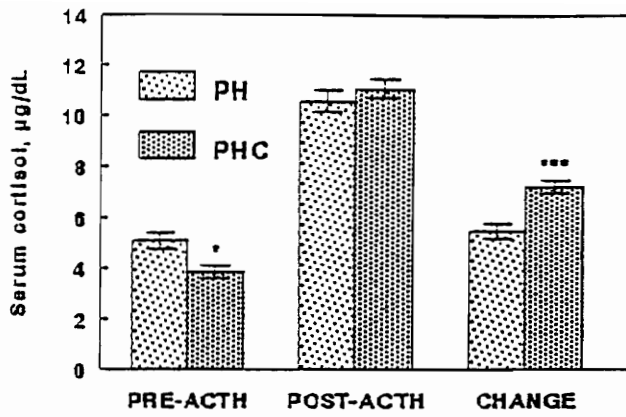


Figure 6. Influences of diet (upper), weaning method (middle), and gender (lower) on pre-ACTH serum cortisol concentrations and the response of serum cortisol to ACTH. Symbols indicate p values as follows: + ($p < .10$), * ($p < .05$), ** ($p < .01$), *** ($p < .001$).

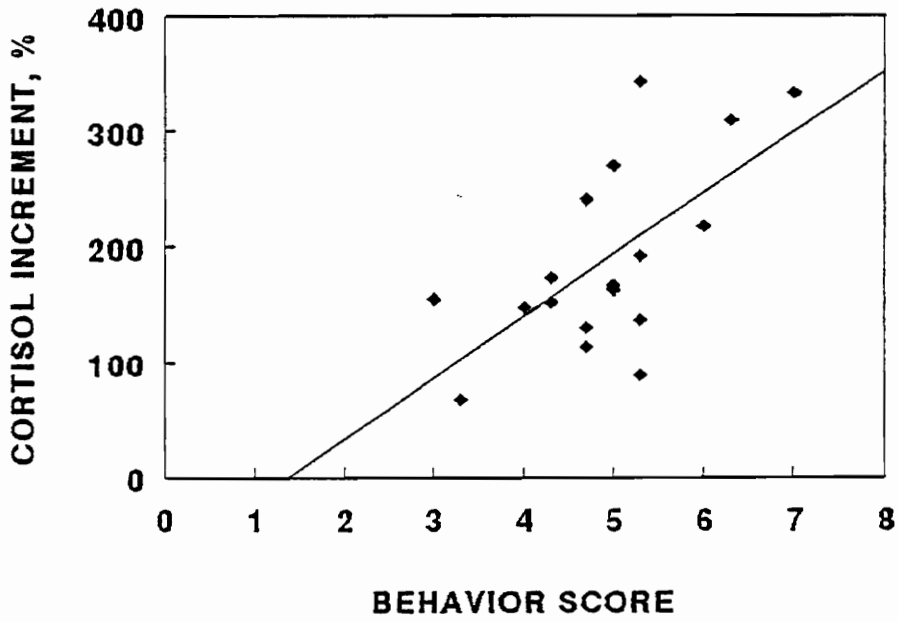


Figure 7. Positive correlation of ACTH response and behavior scores, $p = .0045$, $r = .637$

Equation: $INCREMENT = 53.0(BEHAVIOR SCORE) - 72.5$

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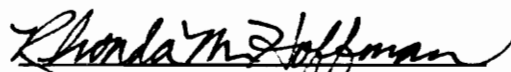
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