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Title: THE EFFECTS OF DIETARY PROTEIN ON REPRODUCTION IN PONY MARES

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Three levels of dietary crude protein (8.6, 11.4, and 17.2) were fed to 24 pony mares to determine the effects on the estrous cycle, conception rate, and serum progesterone concentrations. Mares were allotted into three treatment groups designated as low protein (LP), medium protein (MP), and high protein (HP). Following estrous synchronization with progesterone and prostaglandin $F_{2\alpha}$, dietary protein at the levels investigated, did not affect ($P > .05$) estrous synchronization, length of estrus, the number of days to ovulation, or the number of inseminations per conception. Conception rate following two cycles tended ($P > .05$) to be higher in the MP (87%) group than the LP (50%) or HP (57%) fed groups. Serum progesterone concentrations were affected ($P < .05$) by diet with levels of 6.5 ± .8, 7.9 ± .8, and 10.3 ± .8 ng/ml progesterone (least squares means ± SE) in the LP, MP, and HP fed groups respectively. Serum progesterone on days 4, 8, 12, and 16 post-ovulation tended ($P = .12$) to be higher in pregnant (9.2 ± .7 ng/ml) than non-pregnant (7.3 ± .7 ng/ml) mares. A pregnancy status X day interaction ($P < .01$) indicated higher serum progesterone in pregnant vs non-pregnant mares, respectively, on day 8 (14.3 ± 1.3 vs 9.0 ± 1.2 ng/ml) and day 16 (6.1 ± 1.3 vs 1.4 ± 1.3 ng/ml).

During the course of this reproduction trial, 9 of 16 pony mares fed a pelleted ration containing 60% ryegrass straw developed clinical symptoms of ryegrass staggers, including tremors, incoordination and tetany. Blood parameters remained normal and no gross pathological changes were found. Penicillium cyclopium was isolated in both the feed and feces of the ponies. This neurological disorder has previously only been reported in sheep and cattle feeding upon dry, closely cropped ryegrass pastures. Removing the ryegrass straw from the diet resulted in marked improvement within 2 weeks and complete recovery within 2 months.

# THE EFFECTS OF DIETARY PROTEIN ON REPRODUCTION IN PONY MARES

by

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# THE EFFECTS OF DIETARY PROTEIN ON REPRODUCTION IN PONY MARES

# CHAPTER I

## INTRODUCTION

The horse industry of today has developed into a multibillion dollar-a-year business. This can be clearly seen as the sales of individual horses of many breeds are selling over the million dollar mark, million dollar stallion syndications are becoming common place, and large business firms have begun to invest heavily in the equine industry. However, within this billion dollar industry resides an extremely large and influential group of horsemen known a "pleasure horsemen" and "back-yard breeders". Although individually they impose a less striking economic picture, together they represent the major population of the equine business (Morgan, 1981).

Inflation and economic pressure has forced the total cost of feeding, maintaining, and breeding horses to an all time high. Horsemen can no longer afford to utilize the "hit and miss" approach to equine production. To horse breeders it has become imperative that both mares and stallions perform at maximum efficiency. A study done by Merkt et al., 1979, demonstrates that over the last 150 years, equine reproductive efficiency has not increased at all. Foaling percentages still remain within the 50-60% range. Proper nutrition is a key ingredient to successful breeding management. Unfortunately, nutrition is often the most overlooked and abused factor in equine production.

It has long been known that dietary energy is a major critical factor in reproduction as diets deficient or excessive in energy have

resulted in impaired reproductive performance. This has been demonstrated in swine (Gossett and Sorensen, 1959; Frobish, 1970), sheep (Allen and Lamming, 1961), cattle (Dunn et al., 1969), and in horses (Henneke et al., 1981). It has not been until fairly recently that research has revealed the significance of proper dietary protein as it relates to reproduction. Diets deficient in protein content have led to decreased fertility in swine and fish (Adams et al., 1960; Boaz, 1962; Dahlgren, 1981), anestrus in sows (Hawton and Meade, 1971; Svajgr et al., 1972), embryo mortality in rats (Nelson and Evans, 1953; Giannina and Leathem, 1974; Henricks and Bailey, 1976), and lowered serum progesterone in heifers, rats, and mice (Hill et al., 1970; Giannina and Leathem, 1974; Henricks and Bailey, 1976; Rattner et al., 1978, 1979). It has also been demonstrated that excessive amounts of dietary protein can be detrimental to reproduction. Studies utilizing mice and rats (Knapka et al., 1977; Saitoh and Takahashi, 1977) have shown an increase in embryo mortality. In diary cattle (Jordan and Swanson, 1979) excessive protein resulted in lowered conception rates, and in chickens (Patel and McGinnis, 1977) decreased the hatchability of eggs.

Very little work has been done with larger domestic animals in regard to the relationship between dietary protein and reproduction. It is therefore the intent of this study to investigate the importance of dietary protein concerning estrus and reproduction in pony mares. The objectives of this trial were to determine if deficient or excessive dietary protein affected: 1) estrous synchronization;

2) length of estrus; 3) number of days to first ovulation; 4) number of inseminations necessary for conception; 5) conception percentages; and 6) serum progesterone concentrations.

During the course of this study, 9 pony mares developed a condition which is suspected to have been ryegrass staggers. Ryegrass staggers (RGS) is a neurological disorder which is associated with animals grazing on closely cropped dry pastures in which ryegrass is the dominant herbage (Gallagher et al., 1977; Lanigan et al., 1979; Shreeve et al., 1979). Until recently, very little was known about what caused the RGS syndrome. From several recent studies (DiMenna et al., 1976; Mantle et al., 1978; Lanigan et al., 1979; Shreeve et al., 1979), it appears that several soil fungi, tremorgenic strains of Penicillia, are the causative agents of RGS (Parle, 1976; DiMenna and Mantle, 1978; Shreeve et al., 1978). The latter portion of this paper is a clinical report devoted to describing the RGS syndrome resulting from animals being fed a processed forage rather than on grazed pastures.

# CHAPTER II

## The Effects of Dietary Protein on Reproduction in Pony Mares [1]

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## SUMMARY

Three levels of dietary crude protein (8.6, 11.4, and 17.2) were fed to 24 pony mares to determine the effects on the estrous cycle, conception rate, and serum progesterone concentrations. Mares were allotted into three treatment groups designated as low protein (LP), medium protein (MP), and high protein (HP). Following estrous synchronization with progesterone and prostaglandin $F_{2\alpha}$, dietary protein at the levels investigated, did not affect ($P > .05$) estrus synchronization, length of estrus, the number of days to ovulation, or the number of inseminations per conception. Conception rate following two cycles tended ($P > .05$) to be higher in the MP (87%) group than the LP (50%) or HP (57%) fed groups. Serum progesterone concentrations were affected ($P < .05$) by diet with levels of $6.5 \pm .8$, $7.9 \pm .8$, and $10.3 \pm .8$ ng/ml progesterone (least squares means $\pm$ SE) in the LP, MP, and HP fed groups respectively. Serum progesterone on days 4, 8, 12, and 16 post-ovulation tended ($P = .12$) to be higher in pregnant ($9.2 \pm .7$ ng/ml) than non-pregnant ($7.3 \pm .7$ ng/ml) mares. A pregnancy status X day interaction ($P < .01$) indicated higher serum progesterone in pregnant vs non-pregnant mares, respectively, on day 8 ($14.3 \pm 1.3$ vs $9.0 \pm 1.2$ ng/ml) and day 16 ($6.1 \pm 1.3$ vs $1.4 \pm 1.3$ ng/ml).

# INTRODUCTION

Diets deficient in protein content have led to decreased fertility in swine and fish (Adams et al., 1960; Boaz, 1962; Dahlgren, 1981), anestrus in sows (Hawton and Meade, 1971; Svajgr et al., 1972), embryo mortality in rats (Nelson and Evans, 1953; Giannina and Leathem, 1974; Henricks and Bailey, 1976), and lowered serum progesterone in heifers, rats, and mice (Hill et al., 1970; Giannina and Leathem, 1974; Henricks and Bailey, 1976; Rattner et al., 1978, 1979). It has also been demonstrated that excessive amounts of dietary protein can be detrimental to reproduction. Studies utilizing mice and rats (Knapka et al., 1977; Saitoh and Takahashi, 1977) have shown an increase in embryo mortality. In dairy cattle (Jordan and Swanson, 1979), excessive protein resulted in lowered conception rates, and in chickens (Patel and McGinnis, 1977) it has been reported to decrease the hatchability of eggs.

Very little work has been done with larger domestic animals in regard to the relationship between dietary protein and reproduction. The intent of this study is to investigate the importance of dietary protein concerning estrous and early pregnancy in pony mares. The specific objectives of this trial were to determine if deficient or excessive dietary protein affected; 1) estrous synchronization, 2) length of estrus, 3) number of days to ovulation, 4) number of inseminations per conception, 5) conception percentages, and 6) serum progesterone concentrations.

## MATERIALS AND METHODS

In this trial, 24 pony mares, ranging in age from 2 to 20 yr, were utilized to evaluate the effects of deficient, maintenance, or excessive amounts of dietary protein on reproduction (NRC, 1973). The pony mares were randomly allotted into three treatment groups as designated by the diets received. The groups were designated as low protein (LP), medium protein (MP), and high protein (HP), with dietary crude protein calculated at 8.0, 12.0, and 16.0%, respectively. All animals were maintained by groups in 16 X 40 m outdoor paddocks. The ponies were placed in individual tiestalls twice daily at 0700 and 1600 h for 2 h each session for feeding and handling. Animals were weighed and daily feed intake was calculated for individual maintenance. Water and a trace mineralized salt block were accessible free choice. All experimental diets were processed into .45 cm pellets to alleviate feed refusal and control individual daily intake. All three diets were calculated to be isocaloric containing 2.2 Mcal/kg digestible energy with the only differing factor being the crude protein content. Diet ingredients and composition are shown in tables 1 and 2, respectively. Perennial ryegrass straw was used as the primary ingredient due to its low crude protein value and its availability in the local area. All animals were fed the experimental rations for 3 wk prior to the start of the trial to allow the mares to adjust to the diets and feeding regimen.

All mares were teased daily while in the individual tiestalls. Mares were teased both from the front and back with a vigorous pony

TABLE 1. PERCENTAGES OF INGREDIENTS OF PELLETED DIETS FED TO PONY MARES

| Ingredient | International Ref. No. | Diet [a] LP | MP | HP |
|---|---|---|---|---|
| Per. Ryegrass Straw (lolium perenne) | - - - - | 62.5 | 60.5 | 48 |
| Molasses (beet) | 4-00-668 | 7 | 7 | 7 |
| Soybean meal | 5-04-604 | -- | 12 | 20 |
| Corn | 4-02-985 | 20 | 10 | -- |
| Beet pulp | 4-00-669 | 10 | 10 | -- |
| Alfalfa (midbloom) | 1-00-063 | -- | -- | 25 |
| Dicalcium phosphate | - - - - | .5 | .5 | -- |

[a] Diets, LP, MP, and HP, indicate low, medium, and high dietary crude protein, respectively (NRC, 1973), 100% dry matter basis.

TABLE 2. COMPOSITION OF PELLETED DIETS FED TO PONY MARES

| Item | Diet [a] | | |
|---|---|---|---|
| | LP | MP | HP |
| Crude protein (%) | 8.6 | 11.4 | 17.2 |
| Acid detergent fiber (%) | 30.4 | 30.2 | 28.4 |
| Neutral detergent fiber (%) | 53.1 | 50.6 | 45.2 |
| Ca (%) | .5 | .4 | .6 |
| P (%) | .3 | .3 | .3 |

[a] Diets, LP, MP, and HP, indicate low, medium, and high dietary crude protein, respectively (NRC, 1973). Digestible energy calculated at 2.2 Mcal/kg.

stallion to detect signs of estrus. Mares were palpated rectally every other day during diestrus and daily from the first day of estrus until ovulation was detected. This was to insure that all the mares had at least one normal ovulatory cycle before assigned to treatment. The estrous synchronization regimen utilized was as described by Holtan et al., 1977, except injections of human chorionic gonadotropin (hCG) were not used. To begin the trial, all mares from the three feed groups were randomly selected into groups of eight to begin the estrous synchronization treatment. At four day intervals each of the three groups of selected mares began treatment. This was done to alleviate the possible overuse of the pony stallion. The synchronization program consisted of intramuscular injections (IM) on days 0 through 10 of 50 mg of progesterone suspended in sesame seed oil. On day 7 of treatment, 2.5 mg IM of Prostaglandin $F_{2\alpha}$ was administered. Mares were artificially inseminated with 500 X $10^6$ cells of fresh raw semen every other day from the first or second day of heat until ovulation through two complete cycles.

Blood samples were taken from the jugular vein on days 4, 8, 12, and 16 post-ovulation. After allowing a clot to form at room temperature, serum was separated by centrifugation and then frozen until analysis. Radioimmunoassay of progesterone was a modification of a procedure previously in use (Koligian and Stormshak, 1976). Serum was extracted with 3 ml diethyl ether and the solvent and organic phases separated using a dry ice-methanol bath. The antiserum utilized was anti-progesterone-11-BSA[4] with binding of 38% (Gibori et al., 1977). Individual samples were corrected for recovery using an

[4] Anti-progesterone No. 337. Provided by G. D. Niswender.

external standard, overall recovery was 93.5%. Within-assay coefficient of variation was 13.0% on a sample that contained 3.2 $\pm$ .2 ng/ml (n = 8). Between-assay coefficient of variation was 13.2% on samples that contained 9.6 $\pm$ .4 ng/ml (n = 9). Sensitivity was 10 pg progesterone per tube or .5 ng/ml.

Reproductive parameters monitored were days to first estrus, length of estrus, days to first ovulation, and the number of inseminations per conception. These parameters were analized using a one way analysis of variance. Conception rates were monitored through the two cycles and analized using a Chi square analysis procedure (Hayslett, 1968). Serum progesterone data were compared utilizing the least squares means analysis of variance (Harvey, 1975).

## RESULTS AND DISCUSSION

During the three week adaption period prior to the start of the trial, all mares adjusted to the pelleted diets. After the synchronization program was completed, one mare from the high protein group (HP) and two mares from the low protein (LP) were removed from the trial due to failure to return to estrus, one mare from the HP group was diagnosed as having a false pregnancy after being bred during the first heat cycle post-synchronization and was considered not pregnant in the data analysis.

Dietary protein did not affect ($P > .05$) general reproductive parameters between the three groups following estrous synchronization (table 3). The results of the parameters monitored were similar to those found by Holtan et al., 1977. In none of the categories did any dietary group appear to have a positive or detrimental relationship to dietary protein and reproduction.

The effects of dietary protein on conception tended to favor the MP fed group (table 3). However, the effects were not significant ($P > .05$). It can be seen that the MP fed group had what appears to be a higher conception percentage (87%) as compared to the LP or HP fed groups (50 and 57%, respectively). It must be noted that the sample numbers were small in this trial and any inference as to the effect of diet on conception rate are highly speculative. The tendency to affect conception rates have been reported in several previous studies. Both Adams et al., (1960), and Boaz, (1962), noted that sows on a low protein diet had lower conception rates than sows

TABLE 3. EFFECTS OF DIETARY PROTEIN ON REPRODUCTIVE PARAMETERS FOLLOWING ESTROUS SYNCHRONIZATION.

| Item | Diet [a] LP | MP | HP |
|---|---|---|---|
| Days from end of treatment to estrus | 6.0 ± 3.0[b] | 6.6 ± 2.7 | 3.3 ± .7 |
| Days from end of treatment to ovulation | 11.3 ± 2.9 | 11.8 ± 2.4 | 9.1 ± .5 |
| Length of estrus | 5.3 ± 1.0 | 5.0 ± .8 | 5.7 ± .7 |
| No. of inseminations | 2.8 ± .5 | 2.6 ± .4 | 3.1 ± .3 |
| % pregnant (cycle 1) | 50.0 | 75.0 | 42.8 |
| (cycle 2)[c] | 50.0 | 87.0 | 57.0 |

[a] LP, MP, and HP represent low (8.6%), medium (11.4%), and high (17.2%) dietary crude protein.

[b] Means ± SE. No difference ($P > .05$) between diets for any of the parameters.

[c] Cumulative conception percentages following two breeding cycles.

on adequate protein diets. Hill et al., (1970) found that beef heifers on restricted protein diets had significantly lower conception rates than heifers on adequate diets. A study utilizing dairy cattle (Jordan and Swanson, 1979), found that excessive dietary crude protein reduced conception percentages.

Analysis of serum progesterone data (Harvey, 1975), indicated concentrations were affected ($P < .05$) by dietary protein levels. Serum progesterone concentrations (least squares means SE) were $6.5 \pm .8$, $7.9 \pm .9$ and $10.3 \pm .8$ ng/ml in the LP, MP, and HP fed froups, respectively, when data were combined over days and pregnancy status (pregnant plus non-pregnant). These data indicate a positive linear relationship between dietary crude protein and progesterone levels. This data is supported by several previous studies in which rats (Giannina and Leathem, 1974; Henricks and Bailey, 1976) and mice (Rattner et al., 1979) on protein deficient diets had lower progesterone concentrations than animals on adequate protein diets. However, the apparent reduced conception in both the LP and HP fed groups is difficult to interpret in relation to absolute progesterone differences. Jordan and Swanson (1979) utilizing dairy cattle, reported just the opposite effect with higher crude protein resulting in lowered progesterone concentrations and reduced conception. Progesterone concentrations over days 4, 8, 12, and 16 post-ovulation were best described by a quadratic equation, similar to the work of Squires et al., (1974) and Holtan et al., (1975). There was no ($P > .05$) diet X day

interaction indicating concentrations of progesterone increased and decreased in a similar pattern for all the diets although retaining the main diet effect differences (figure 1).

Serum progesterone concentrations in pregnant mares (9.2± .7 ng/ml) tended to be higher ($P = .12$) than non-pregnant mares (7.3 ± .7 ng/ml). The diet X pregnancy status (pregnant or non-pregnant) interaction was not significant indicating the tendency for pregnant mares to have higher progesterone concentrations than non-pregnant mares, over all diets, was again a positive linear relationship as for diet alone. It can be seen (figure 2) that the pregnancy status X day interaction ($P < .01$) was attributable to higher progesterone concentrations in pregnant vs non-pregnant mares, respectively, on day 8 (14.3± 1.3 vs 9.0 ± 1.2 ng/ml) and day 16 (6.1± 1.3 vs 1.4 ± 1.3 ng/ml). This finding of a serum progesterone difference on day 8 is not in agreement with previous studies (Squires et al., 1974; Holtan et al., 1975). In both of these studies, serum progesterone concentrations were found to be elevated on day 8 in both pregnant and non-pregnant mares, but the two groups did not differ ($P > .05$). However, on days 14 to 16, both Squires et al., (1974) and Holtan et al., (1975) found significant increases in serum progesterone concentrations in pregnant vs non-pregnant mares.

These data indicate that short term dietary crude protein differences in the mare of approximately 8, 12, and 17% do not affect general reproductive parameters (estrus and ovulation), but do affect progesterone concentrations which in turn may be related to differences in conception rate. It would be difficult to conclude from this

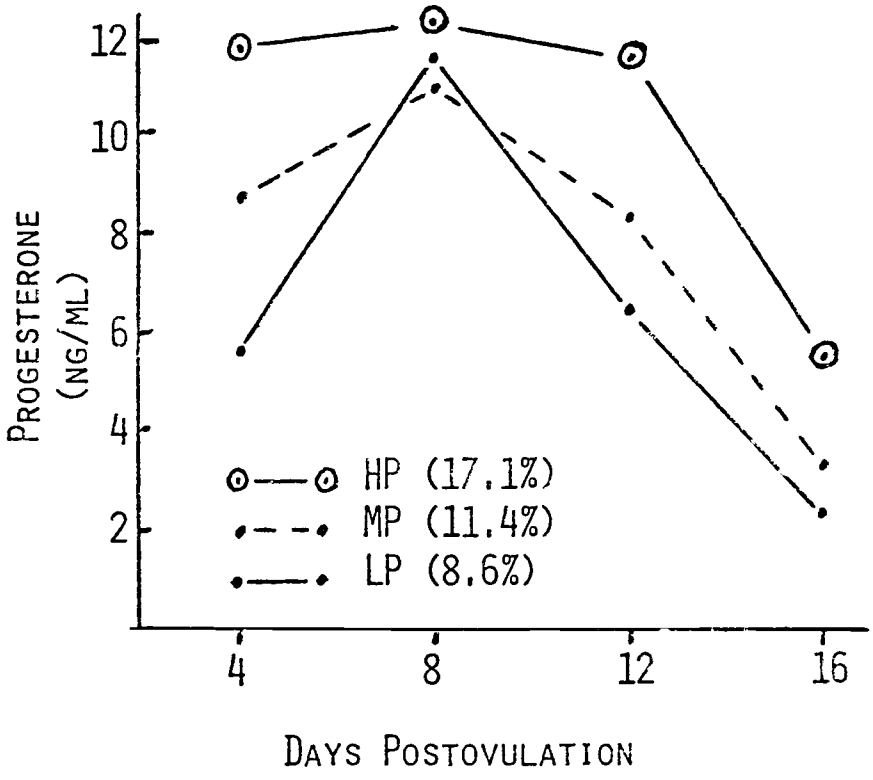


FIGURE 1. Serum progesterone in pony mares fed 8.6% (LP), 11.4% (MP), or 17.2% (HP) crude protein. Data are least squares means; pooled SE = 1.5. Main effect of day is different ($P < .05$). Diet X day interaction is not different ($P > .05$).

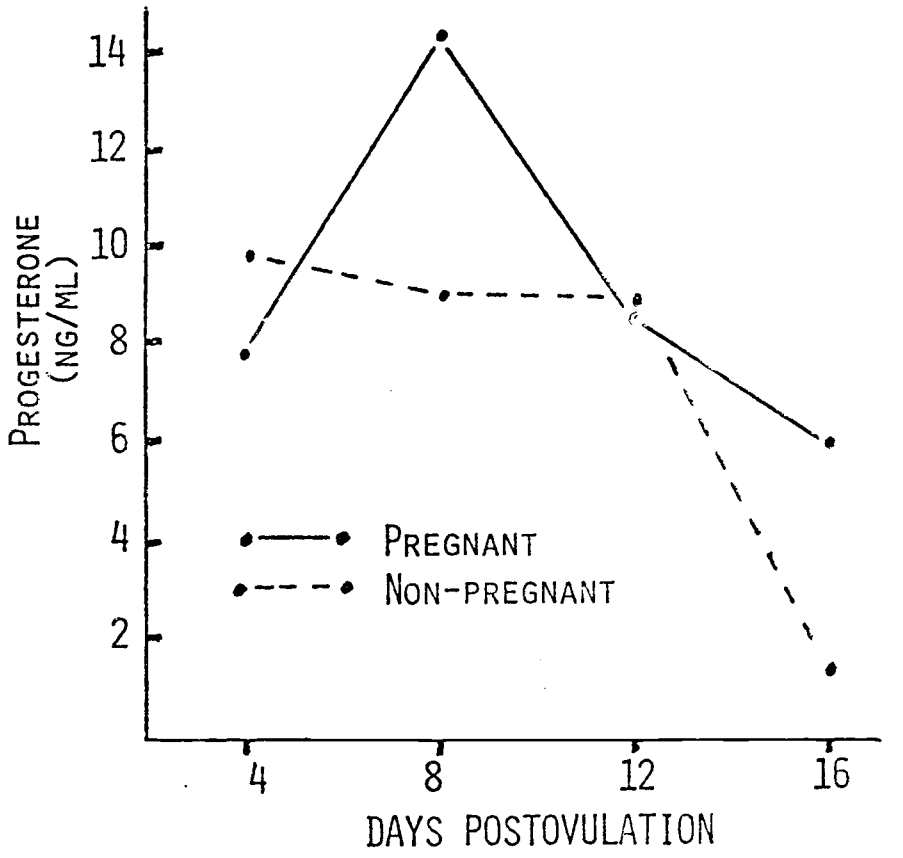


FIGURE 2. Serum progesterone in pregnant and non-pregnant pony mares. Data are least squares means; pooled SE = 1.3. Pregnancy status main effect not different ($P = .12$). Pregnancy status X day interaction was different ($P < .05$), due to difference ($P < .05$) on days 8 and 16.

study whether of not increased dietary crude protein would be beneficial or detrimental to reproduction. These relationships, although preliminary and similar to those found in rats (Henricks and Bailey, 1976), need to be elucidated before firm conclusions and recommendations can be made. If, as it appears, dietary protein affects progesterone levels, how does it affect other important reproductive hormones, such as LH? What are the possible long term effects on such factors as; incidence of embryo mortality, maintenance of pregnancy, and subsequent conception rates? Perhaps as more research is completed, we will gain a greater understanding of how protein quantity as well as quality affects reproduction and hopefully how equine nutrition as a whole affects reproductive efficiency.

# CHAPTER III

## RYEGRASS STAGGERS IN PONIES FED PROCESSED RYEGRASS STRAW[1]

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## A CLINICAL REPORT

In a study pertaining to reproduction, 24 pony mares ranging in ages from 2 to 20 years were allotted to 3 treatment groups and were fed a complete pelleted ration that had perennial ryegrass (Lolium perenne) straw as the primary ingredient. The percentage of ryegrass straw in each ration was: group 1, 45%; group 2, 60%; and group 3, 60%. The intent of the research was to study dietary protein and its effects on the equine estrous cycle and early pregnancy. The ponies were fed the rations for 5 months.

At 3 weeks, behavioral changes were noticed in the ponies in groups 2 and 3. Signs ranged in severity from mild excitability to spastic ataxia, including hypermetria, dysmetria, weaving when standing still, hyperexcitability, and in extreme cases, tetany. Ponies in tetany, if left undisturbed, would recover after a few minutes, regain their feet, and walk off stiffly. Throughout the trial, none of the affected ponies lost weight, Ponies were placed in 1 of 4 clinical categories: (1) nonaffected (no clinical signs), (2) mildly affected (hyperexcitable), (3) moderately affected (hyperexcitable, gait abnormalities), (4) severely affected (hyperexcitable, gait abnormalities in all limbs, and collapsing in tetanic seizures when forced to move). At 3 months, 9 of the 16 ponies in groups 2 and 3 were affected to varying degrees (3 mildly, 3 moderately, and 3 severely). The 8 ponies in group 1 remained clinically normal. During the trial, none of the mildly or moderately affected ponies showed progression of the neurologic dysfunction, nor did any of the severely affected ponies die.

Originally, the signs were hypothesized to be caused by a selenium deficiency, a prevalent nutritional disease in Oregon. Blood samples were taken from all the ponies and tested for glutathione peroxidase (GSH-Px) activity (each molecule of GSH-Px contains selenium and, therefore, determination of blood GSH-Px activity is considered an effective test of selenium status) (Van Vleet, 1980). In addition, blood selenium was measured in one half of the ponies. Both tests revealed no differences between the affected and nonaffected ponies. Blood samples were taken from 2 ponies in group 1, and from 2 moderately affected and 3 severely affected ponies in the other groups. Blood chemical panels (lactate dehydrogenase, glutamic oxaloacetic transaminase, phosphorus, calcium, bilirubin, total protein, albumin, creatinine, blood urea nitrogen, glucose, sodium, potassium, and chloride) were normal (Kaneko, 1980). Packed cell volume, white cell counts, red cell counts, and differential counts also were normal. Paired lumbosacral cerebral spinal fluid samples were taken 1 to 2 months apart from the 3 most severely affected ponies. The only abnormality was a protein concentration ranging from 76 to 138 mg/dl, normal values for horses being less than 70 mg/dl (DeLahunta, 1977).

It was suspected that the feed was contaminated with a tremorgenic mycotoxin, even though ryegrass staggers has been reported only on grazed pastures (Clegg and Watson, 1960; Gallagher et al., 1977; Patterson et al., 1979; Shreeve et al., 1979). Specimens of the ryegrass straw bales used in the trial were collected for bacteriologic analysis. Fecal samples were collected from 2 clinically normal

ponies in group 1 and from 2 moderately and 2 severely affected ponies in groups 2 and 3. Penicillium cyclopium was found in both the ryegrass straw and in fecal samples from the nonaffected and affected ponies. Further efforts to isolate and to purify toxin(s) in the P. cyclopium, or to reproduce the syndrome in the laboratory were not done because of insufficient time, personnel, and funds. It was theorized that only those ponies in groups 2 and 3 were getting sufficient mycotoxin to induce the signs of ryegrass staggers. To test this theory, one of the severely affected ponies was switched to a diet of grain and mixed-grass hay. Within 2 weeks, marked improvement had occurred and recovery was complete after 2 months. Another of the most severely affected ponies was euthanatized and necropsied. There were no gross or microscopic lesions, which is in accord with findings in previous studies (Clegg and Watson, 1960; Munday and Mason, 1969).

Ryegrass staggers is manifested as a neurologic disorder characterized by muscular tremors, locomotor incoordination, and tetany (Dimenna et al., 1976; Gallagher et al., 1977; Shreeve et al., 1978). Animals often appear normal at rest. When incited to move, they have stiff, spastic gaits, exaggerated limb action, muscular spasms, and occasionally tetanic seizures, from which they recover after a few minutes of rest. The condition is reversible, and recovered animals do not appear clinically to suffer any permanent damage from the disorder. Affected animals are frequently in good condition and routine clinical blood chemical analysis reveal no abnormalities (Clegg and Watson, 1960; Shreeve et al., 1978).

Until recently, little was known about the cause of the ryegrass staggers syndrome. From several recent studies (DiMenna et al., 1976; Mantle et al., 1978; Lanigan et al., 1979; Shreeve et al., 1979), it appears that several soil fungi, tremorgenic strains of penicillia, appear to be the causative agents of ryegrass staggers (Parle, 1976; DiMenna and Mantle, 1978; Shreeve et al., 1978). Penicillium cyclopium, P. janthinellum, P. piscarium, and P. canescens (Gallagher et al., 1977; DiMenna and Mantle, 1978; Giesecke et al., 1979; Lanigan et al., 1979) are but a few of the penicillia known to produce tremorgenic mycotoxins that affect livestock. Tremorgenic toxins produced by the aforementioned species include: penitrem A, verrucologen, and funitremogin B. These compounds characteristically induce trembling and other disturbances of the neuromuscular system (Gallagher et al., 1977; Lanigan et al., 1979; Patterson et al., 1979).

In this case, it appears that a chronic form of ryegrass staggers had developed as a result of feeding pellets made from straw contaminated with P. cyclopium. After the ponies were taken off the pelleted rations, they recovered completely.

# CHAPTER IV

## GENERAL CONCLUSIONS

These studies have produced information regarding the effects of dietary protein on reproduction in pony mares, and also information in the area of Ryegrass Staggers Syndrome. Specific conclusions that can be derived from these studies include the following:

1. This study indicated that short term deficiencies or excesses of dietary protein do not affect general reproductive parameters such as length of estrus, number of days to ovulation, or the number of inseminations necessary for conception.

2. Serum progesterone levels are directly affected by the amount of crude protein present in the diet.

3. Pregnant mares have significantly higher serum progesterone levels on days 8 and 16 than do non-pregnant mares.

4. Dietary protein may affect conception percentages.

5. Previous data indicate ryegrass staggers occurring only on grazed pastures, however this study indicated that it is possible to produce the ryegrass staggers syndrome from a harvested forage.

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