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Original Research

Effect of Feeding Hay on Nonesterified Fatty Acids in Appetite-suppressed Pregnant New Zealand White Rabbits

Jesse W Veenstra,[1,*] Adam J Filgo,[2] and Steven C Denham[3]

Pregnant rabbits are a common nonrodent model for reproductive safety evaluation in preclinical drug development. During reproductive toxicology studies, rabbits are prone to decreased food consumption and anorexia. When persistent or severe, this condition can lead to hepatic lipidosis and pregnancy toxemia, which may confound the interpretation of study results. Non-Esterified Fatty Acids (NEFAs) have been used in veterinary production medicine to evaluate the impact of diet on the energy balance of pregnant animals. In the current study, sustained-release buprenorphine was used to suppress the appetite of pregnant New Zealand white rabbits, mimicking the clinical presentation of animals in reproductive toxicology studies. Sequential NEFA evaluations during gestation, along with other clinical endpoints, such as the necessity and duration of veterinary intervention, were used to evaluate the effects of feeding hay and a pelleted diet as compared with a pelleted diet alone. Elevated NEFA levels were directly correlated to litter size, the number of viable fetuses and the number of days on veterinary consult due to severely decreased consumption of pelleted diet. Animals with hay as part of their diet did not require additional diet supplementation as determined by qualitative evaluation of hay intake and adequate fecal output. These data suggest that including hay as a portion of the standard diet benefits pregnant rabbits in laboratory or production settings.

Abbreviations and Acronyms: GD, Gestational day; GLP, Good Laboratory Practices; NEFA, Non-Esterified Fatty Acid; H, hay group; H-AS Hay and appetite suppressed group; NH, no hay group; NH-AS, no hay and appetite suppressed group; SR, sustained release.

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In preclinical drug development, pregnant rabbits are a common nonrodent model for evaluating developmental and reproductive toxicology. Common veterinary concerns with rabbits in reproductive toxicology studies are gastroinstestinal upset and decreased food consumption.[11] At our facility, approximately 75% of requests for veterinary consultation with New Zealand white rabbits in the 12 mo prior to this study were due to decreased food consumption. Sustained decreased food consumption places the animal in a metabolic state of negative energy balance.[30] A prolonged state of negative energy balance in which an animal's caloric intake is not adequate to meet energy demands can lead to a number of pathologic processes such as ketosis and hepatic lipidosis. One of the biomarkers of negative energy balance in animals is the production of Nonesterified Fatty Acids (NEFAs).[7]

When an animal's energy requirements outstrip its easily accessible glycogen stores, the body turns to adipose tissue as its next substrate for energy. When adipose tissue is mobilized for energy, fatty acids are released into the blood where they are reversibly bound to albumin. This complex is referred to as a NEFA.[7] While tissues can use a small amount of the complex, the majority of NEFAs are transported to the liver for further metabolism in the Krebs cycle into usable energy. If the Krebs cycle's ability to oxidize NEFAs within the liver is overwhelmed, NEFAs are alternatively moved into ketone body production or very-low-density lipoprotein (VLDL) synthesis.[7,29,30]

When the metabolic state of energy deficit and thus, breakdown of adipose tissue, is prolonged or severe, the ketone bodies produced are released into the bloodstream and the newly synthesized VLDLs are either secreted or deposited within the liver. The ability of the liver to process VLDLs can become overwhelmed, leading to hepatic lipid deposition. Eventually, the functionality of the hepatocytes, and the liver as a whole, are compromised due to the damage caused by excessive lipid deposition.[9,10,25] This results in a systemically ill animal with even less appetite, compounding the clinical problem.[7] Ketone bodies, which are also elevated during the state of energy deficit, are also associated with decreased appetite in multiple species, further exacerbating inappetence.[2,13] In pregnant rabbit does, decreased food consumption and hepatic lipidosis are risk factors for pregnancy toxemia.[3] Ketoacidosis and severe electrolyte imbalances characteristic of pregnancy toxemia can result in mortality of the fetuses and the dam unless early and aggressive treatments are initiated.[3,28]

NEFAs have also been identified as a biomarker for energy-deficit in pregnant females of other species. In dairy cattle, measurement of NEFA levels is used as a tool in production

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[1]Veterinary Services, [2]Department of Development and Reproductive Toxicology, [3]Bioinformatics Sciences, Charles River Laboratories, Mattawan, Michigan
[*]Corresponding author. Email: Jesse.Veenstra@crl.com

medicine for evaluating energy balance in groups of pregnant animals. The 2 wk prior to the end of gestation have been defined as the opportune period to identify dairy cattle in negative energy balance using serum NEFA levels.[23] Elevated NEFAs are associated with an increased risk for parturition-related diseases in dairy cattle such as metritis, mastitis, and metabolic diseases.[1] NEFAs have also been identified in humans, as a marker of potential energy-related pregnancy complications. Clinical studies determined an increase in maternal NEFAs was directly related to women with intrauterine growth-restricted pregnancies.[24] The NEFA profile of rabbits and other species experiencing food restriction during pregnancy has been described in previous literature;[17,20] however, the impact of hay on energy balance and NEFAs in the pregnant rabbit has not been evaluated. Because NEFA levels have been used as a marker in multiple species[5,12] we tested the use of NEFAs to evaluate animal diets in pregnant rabbits in this study.

As a commercial meat animal, the impact of feed restriction and fasting in pregnant New Zealand white rabbits has been described.[4,20,22] Results indicate decreased food intake in mid and late lactation breeding does can result in decreased production parameters, such as litter size, litter weights, weaning weights, and can increase preweaning mortality among the kits.[4,22] Among breeding animals in rabbit production facilities, changes in the digestive tracts of affected animals has been identified as one of the main causes of mortality.[27]

Digestive health of pregnant rabbits is a key focus of veterinary medicine in a toxicology laboratory setting as well. A common side effect of investigational drug administration in rabbits is anorexia.[11] In addition, shipping stress in time-mated rabbits can result in periods of anorexia which can affect animal health, and potentially confound study data in preclinical drug safety trials. Literature[3,8,28,31] indicates that decreased gastrointestinal motility has been linked with anorexia in rabbits, and that indigestible fiber is a key component in maintaining normal gastrointestinal motility and healthy cecal fermentation. While pelleted diets are often high in fiber, this fiber is ground to a fine particle size to produce the pellets, and these fine particles fail to meet the requirements for healthy cecocolonic motility.[8] Large particles of indigestible fiber promote gastrointestinal motility and are associated with improved health, growth, and production in multiple species that use fermentation as an energy source.[18,32] Traditionally, this fiber is supplied to rabbits as fresh grass or hay.[14,26,28,31] Standard supportive care for animals on veterinary consult for anorexia in our facility starts with offering hay. If the hay is not consumed, other forms of edible enrichment are then used to encourage food consumption and promote gastrointestinal health.

The goal of this study was to evaluate the effects of offering hay without retriction in addition to a pelleted basal lab diet on pregnant rabbits when compared with the basal lab diet alone. Correlations between maternal serum NEFAs, dietary intake, clinical outcomes and results from reproductive focused necropsies were used to evaluate the effectiveness of the hay supplemented diet. An investigational study to evaluate the impact of feeding hay was performed to validate using additions to standard diet in future GLP rabbit reproductive toxicology studies.

Materials and Methods

Animals. Female, time mated New Zealand white rabbits (Charles River Laboratories, Oakwood Cr:KBL(NZW) MI *n* = 30, weight 3.09 to 5.08 kg) were obtained at GD 0. Animals were nulliparous does, 5 to 8 mo of age, mated at the vendor facility and transported to the testing facility after vendor's confirmation of mating. Animals were housed in an AAALAC accredited facility and maintained according to standards in the *Guide for the Care and Use of Laboratory Animals*.[16] The protocol was approved by the IACUC of MPI Research (now Charles River Laboratories). Animals were housed individually in stainless steel cages to allow for individual food consumption measurements to be obtained. Temperature and humidity were maintained between 61 and 72 °F, and 30% to 70% relative humidity according to MPI Research standard operating procedures and lighting was set at a 12hr light:dark cycle. Tap water was supplied without retriction to all animals by using an automatic water system. All animals were offered Lab Diet Certified Rabbit Diet #5322 PMI Nutrition International (17.1% protein, 3.0% fat, 15.9% crude fiber and max 12% moisture). Pelleted food consumption was measured daily.

Study Design. Upon receipt, on GD 0, animals were randomized into 4 study groups, designated as groups NH (no hay *n* = 10), NH-AS (no hay, appetite suppressed *n* = 7), H (hay *n* = 6), and H-AS (hay, appetite suppressed *n* = 7). Group size was determined by animal availability and ability to dual enroll NH group into on-going studies within the testing facility. Randomization was by weight (± 20% of mean body weight), Provantis, the electronic documentation system used for data collection, generated the randomization. In addition to the Lab Diet #5322, H group and H-AS group animals were fed hay without retriction (Oxbow Western Timothy Hay—min 7% crude protein, min 1.5% crude fat, min 32% crude fiber, max 15% moisture) throughout the duration of the study. Animals in the NH and NH-AS were not offered hay or other food enrichment such as fresh produce unless placed on veterinary consult. Food was weighed at initial offering and then weighed daily before and after feeders were filled. Food consumption was calculated by the difference between the amount remaining and the weight after feeders were filled the day prior. For all groups, if daily food consumption was measured at less than 40 g per day, the animals were determined to be anorexic and placed on veterinary consult. This is consistent with the general process for requesting veterinary consultation for rabbits on reproductive toxicology studies within the testing facility. Animals were also placed on veterinary consult if any signs of morbidity or injury were present. Consumption of hay or food enrichment was assessed qualitatively by observing and recording whether the animal was eating any or none of the offering.

Veterinary technicians involved in clinical care were familiar with developmental and reproductive toxicology rabbit studies and applied the same treatment recommendations as for similar studies being run under GLP standards concurrently in the same facility. Planned veterinary treatment of anorexia consisted of offering novel food such as hay, Oxbow Critical Care, and fresh produce to NH groups, Oxbow Critical Care, and fresh produce to H groups. Novel food items included apple slices, baby carrots, celery and Bio Serv Certified Mardi Gras Foraging Mix. Treatment decisions were based on food consumption data, qualitative consumption of hay, qualitative evaluation of fecal output and the animal's interest in produce, if offered. Animals offered hay by study design did not receive additional novel food items unless hay was not being consumed. Qualitative hay consumption was recorded daily. Hay was the first novel food item offered to NH groups and if animals were not eating hay additional novel food items were offered. Animals with multiple days of pelleted food consumption of over 100 grams/day or animals in hay groups eating hay offered had any novel food items removed. Once animals showed adequate appetite, had normal fecal output, adequate

body condition and were otherwise healthy, they were removed from veterinary consultation. Definition of days on veterinary consultation included all days when animals were under the care of the veterinary staff, which included evaluation, monitoring, and treatments. Veterinary consultation also encompasses days when there were no treatments administered but animals were being monitored by the veterinary staff. In this study veterinary treatments included any novel food items offered and topical treatments for skin lesions.

On GD 7 all animals in H-AS and NH-AS groups were dosed once with 0.3 mg/kg of Buprenorphine SR (ZooPharm, Windsor CO, 3mg/mL) subcutaneously in the dorsal region to suppress appetite.[6,19] This approach mimicked international guidelines for a typical developmental and reproductive toxicology study design in which dosing of investigational compounds usually starts on GD 6 or 7 so that fetuses are exposed to the compound during organogenesis.[15] On GD 6, 10, 14, 18, 22 and 26, blood was collected in a serum separator tube (1mL) from the jugular vein for analysis of NEFAs. Blood samples were collected in the morning just prior to morning feed offerings in an effort to capture peak NEFA values.[23] Animals were not fasted overnight prior to blood collection. Samples were analyzed by an automated clinical chemistry analyzer (AU2700, Beckman Coulter, Brea CA). Assay performance standards: manufacturer dynamic range: 0.01 to 2.5 mEq/L, 2 levels of QC material intraassay CV 0.3% to 0.95%, during internal validation with rabbit samples intraassay CV 3.47%, intraassay CV on 2 levels of QC material 0.00% to 1.16%. Bodyweights were measured on GD 0, 6, 10, 13, 16, 19, 21, 25 and 29. All animals were evaluated daily for morbidity, mortality, injury, and availability of food and water. On GD 29 all animals were euthanized by IV administration of euthanasia solution and submitted for gross necropsy. The uterus for each animal was evaluated for viable and nonviable fetuses, early and late resorptions in each horn, and total implantations were recorded. All fetuses were euthanized by intraperitoneal administration of euthanasia solution. Uteri that appeared nongravid were placed in 10% ammonium sulfide solution for detection of implantation sites; any foci detected were considered early resorptions.

Pooled fecal samples were taken from each group on GD 6, GD8 and GD 22 and frozen between -60 and -90 °C for future microbiome research.

Statistical analysis. Statistical analysis was performed using SAS software (Cary, MC). Significance was determined by a P value less than 0.05, with P values less than 0.05 reported. NEFA analysis by group was conducted by repeat measures of analysis of variance (RMANOVA). Linear regression models with type I SS and Pearson Correlation values were used to evaluate impact of litter sizes and total implants. Linear regression models with type 3 fixed effects were used to evaluate days on consult, and days on treatment compared with NEFAs. These variables, as well as litter size, total implants, days on consult and days on treatment, were also evaluated independent of group to better understand impact of pregnancy on NEFAs. Analysis of food consumption on NEFAs was evaluated using Type 3 fixed effects and linear regression with least square means. The effect of group, independent of NEFAs, on days on consult or days of treatment was tested using Mantel-Haenszel χ^2 likelihood.

Results

Food Consumption. Within 1 d after buprenorphine SR administration, 9 of 14 animals (64%) dosed had food consumption drop to less than 40 g/day, initiating veterinary consultation. Of animals in groups not dosed with buprenorphine SR, food consumption values of 4 of 15 animals (27%) dropped drop below 40 g/day in the same time period. One animal in the NH group had been previously been on veterinary consult for inappetence since arrival. A T-test comparing the change in food consumption between days 6 and 8 of animals dosed with buprenorphine SR on day 7 and those not dosed with buprenorphine SR was significant ($P < 0.05$). Groups were not statistically different based on linear regression models. Average food consumption data is presented in Figure 1. Apparent dips were noted on GD 15 and GD 27; these were due to cage rotation and a shortened interval for food consumption data collection respectively. However, these were not found to be statistically significant.

NEFA Analysis. When NEFAs were evaluated by group using RMANOVA, no significant differences were observed among groups. No significance could be detected between food consumption and groups or food consumption associated with NEFA values using a linear model, whether or not effect of group was fixed (Figure 2).

However, when NEFA values were analyzed relative to clinical outcomes irrespective of group and diet, significant differences were detected. Clinical outcomes of days on veterinary consultation were evaluated both by the intervals prior to NEFA sample and by intervals after sample collection. Elevated NEFAs were positively correlated with days on consult during the interval prior to collection (Figure 3) at GD 14 ($P < 0.0001$), GD 18 ($P < 0.0001$) and GD 22 ($P = 0.0026$). The number of days on veterinary consult in the interval after NEFA sample collection (Figure 4) correlated positively at GD 10 ($P < 0.0001$), GD 14 ($P < 0.0001$), GD 18 ($P < 0.0001$) and GD 22($P = 0.0060$). At GD 6 P value ($P = 0.0503$) did not reach statistical significance but followed the trend seen at most NEFA time points with regard to days on consult after sample collection.

Evaluating necropsy data, elevated NEFAs were positively correlated with the number of total implants at GD 18 ($P = 0.0435$), GD 22 ($P = 0.004$) and GD 26 (0.0065). Similar results were seen for viable fetuses (Figure 5) with positive correlations at GD 18 ($P = 0.0297$), GD 22 ($P = 0.0003$) and GD 26 (0.0018).

Clinical endpoints. Of the groups dosed with buprenorphine SR, 9 of 14 animals developed scabbed areas in the dorsal region in the area of the dose. In the H-AS group 4 of 7 developed scabbed areas with 3 being severe enough to be placed on veterinary consult and in the NH-AS group 5 of 7 developed scabbed areas with 4 of the 7 placed on veterinary consult for the condition. Most lesions developed 7 to 14 d subsequent to the dose. Skin lesions in one animal in the NH-AS group were severe enough to require treatment on GD 24 to 27 with a topical antibiotic ointment (neomycin, polymyxin, bacitracin). One animal in H-AS and NH-AS each, were on consult concurrently for anorexia and skin lesions (Figure 6).

Clinical outcomes irrespective of NEFA analysis by groups revealed significant difference in days on consult between groups, with the group receiving hay and no appetite suppression having the fewest number of days on consult ($P < 0.0001$). In evaluating total days of treatment compared with all study days (Figure 7), significance was detected ($P < 0.0001$) across groups irrespective of whether days of treatment or consult due to skin lesions were or were not included in the analysis. Animals already receiving hay as part of their diet did not require any additional treatment for their decreased food consumption of pelleted basal lab diet (Figure 7).

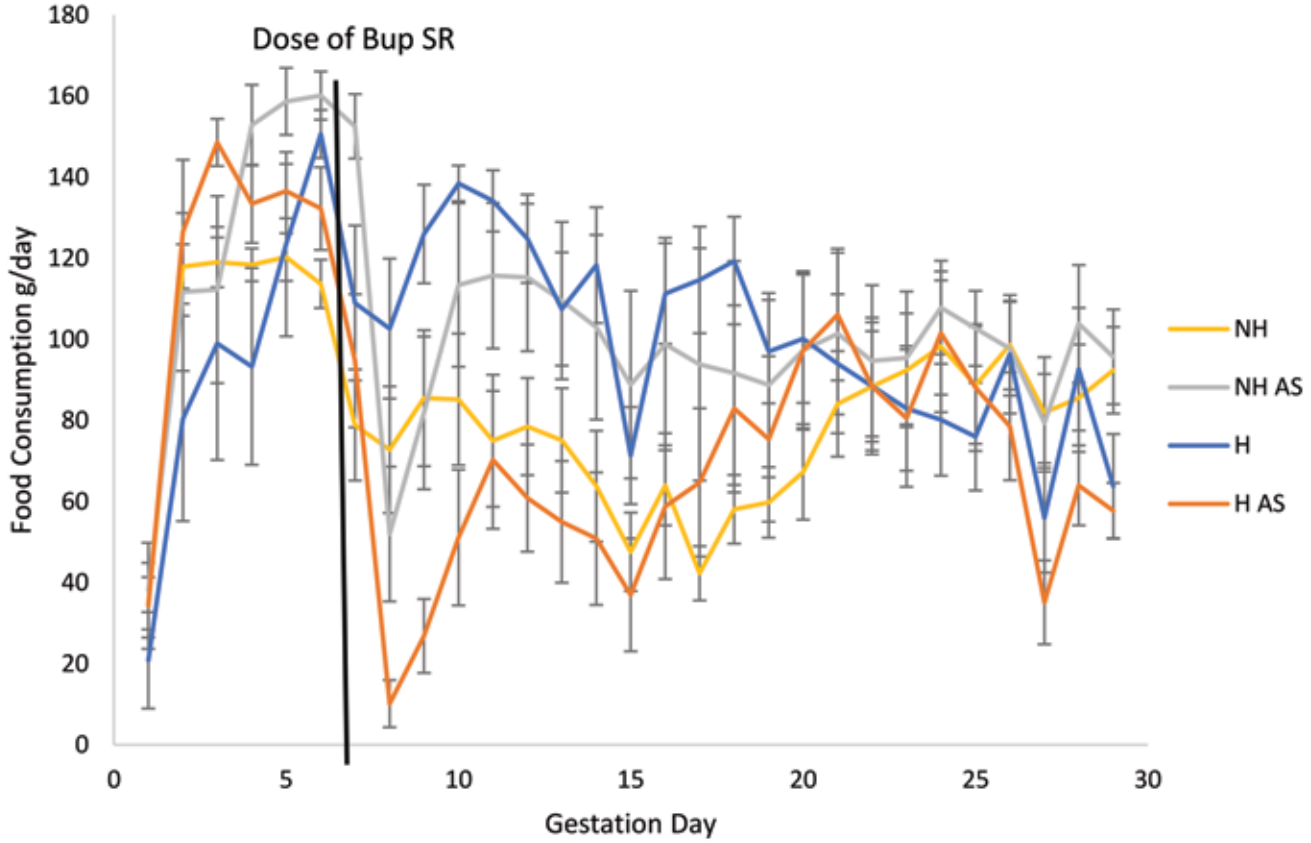

Figure 1. Average daily food consumption (g/day) of pelleted diet by group. Drop in food consumption after buprenorphine SR can be seen on GD 7. Data points show the mean ± S.E.M.

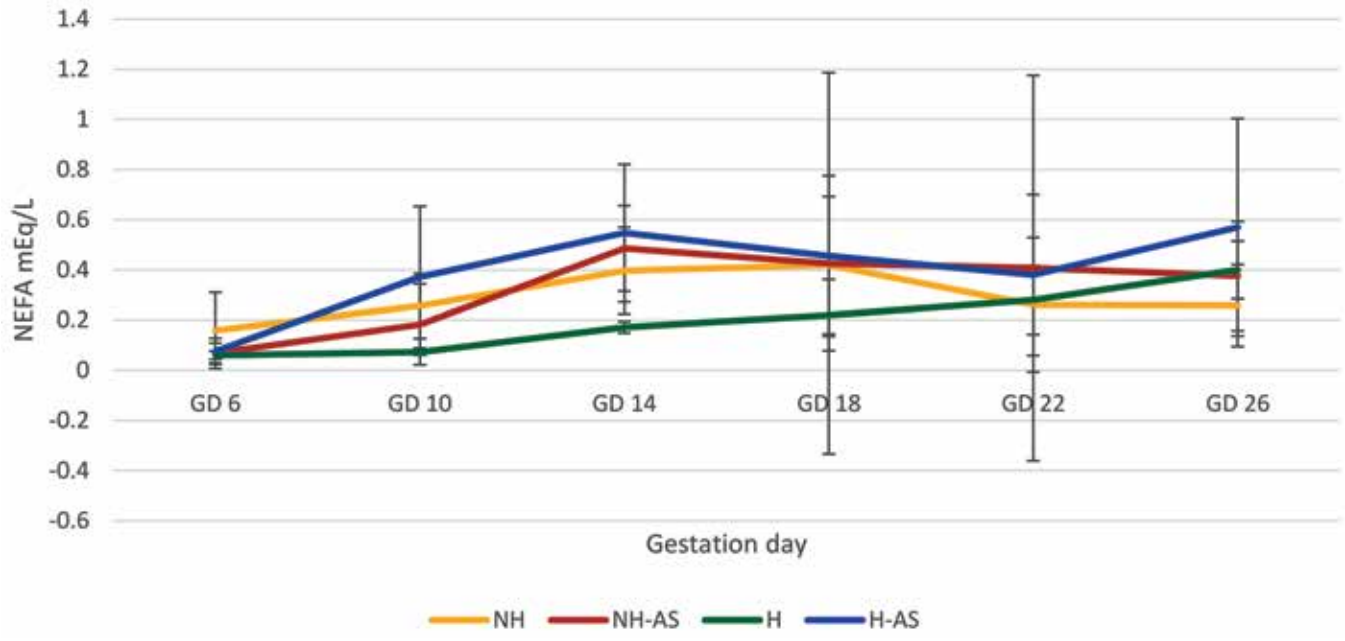

Figure 2. NEFAs (mEq/L) by group. Data points show the mean ± S.E.M. No significant diffreences were detected between groups.

Discussion

Food Consumption. The significant drop in food consumption after dosage with buprenorphine SR when compared with control groups fed the same diet confirm that buprenorphine SR was effective in producing a model of appetite suppression in rabbits. Further evaluation of the skin lesions caused by the buprenorphine SR is necessary to determine if buprenorphine or the sustained release formulation caused this complication before repeating this model of appetite suppression in rabbits. Because hay was offered to both

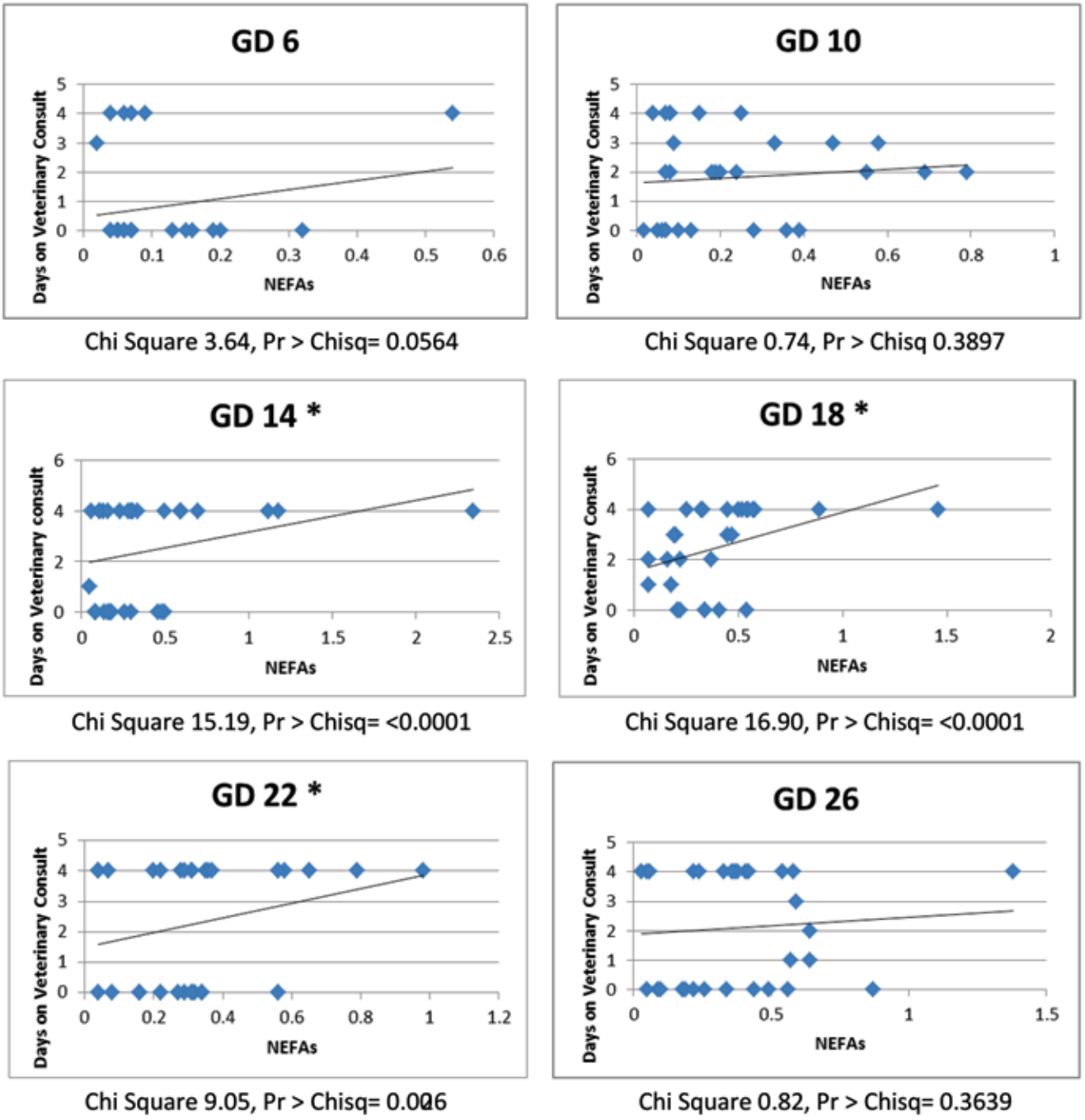

Figure 3. NEFA concentrations are plotted against thee number of days on veterinary consult within the 4 d interval preceding sample collection. Linear regression modeling results, χ^2 values and Pr > Chisq values are shown, with significant effects in bold (*,Pr > Chisq less than 0.05). During midgestation (GD 14-22) the number of days on consult prior to sample collection correlated to elevated NEFA levels.

hay groups without retriction or quantitative measurement, food consumption values of the H and H-AS groups were potentially underreported, making statistical interpretation of quantitative food consumption between hay and no hay groups difficult. The subsequent lower food consumption values of animals fed hay as part of the study design resulted in a high number of animals in the hay groups being placed on veterinary consultation based on predetermined thresholds. Qualitative evaluation of hay intake was reported daily for all animals receiving hay and used for determining clinical endpoints in addition to food consumption and clinical presentation.

NEFAs. When comparing results from this study to published literature on NEFAs in pregnant rabbits,[17,20] the key differences in study design were that this study used an appetite suppressing event rather than food restriction, and that hay was evaluated as part of the diet. Manchietti and colleagues[20] demonstrated that NEFAs were affected by food restriction during late gestation, but not during early and midgestation. While late gestation is reported to be more sensitive to food restriction, gestation day 6 was chosen in this study as the day of the appetite suppression event to follow typical reproductive toxicology study designs[15] and mimic the clinical presentation seen on these studies.

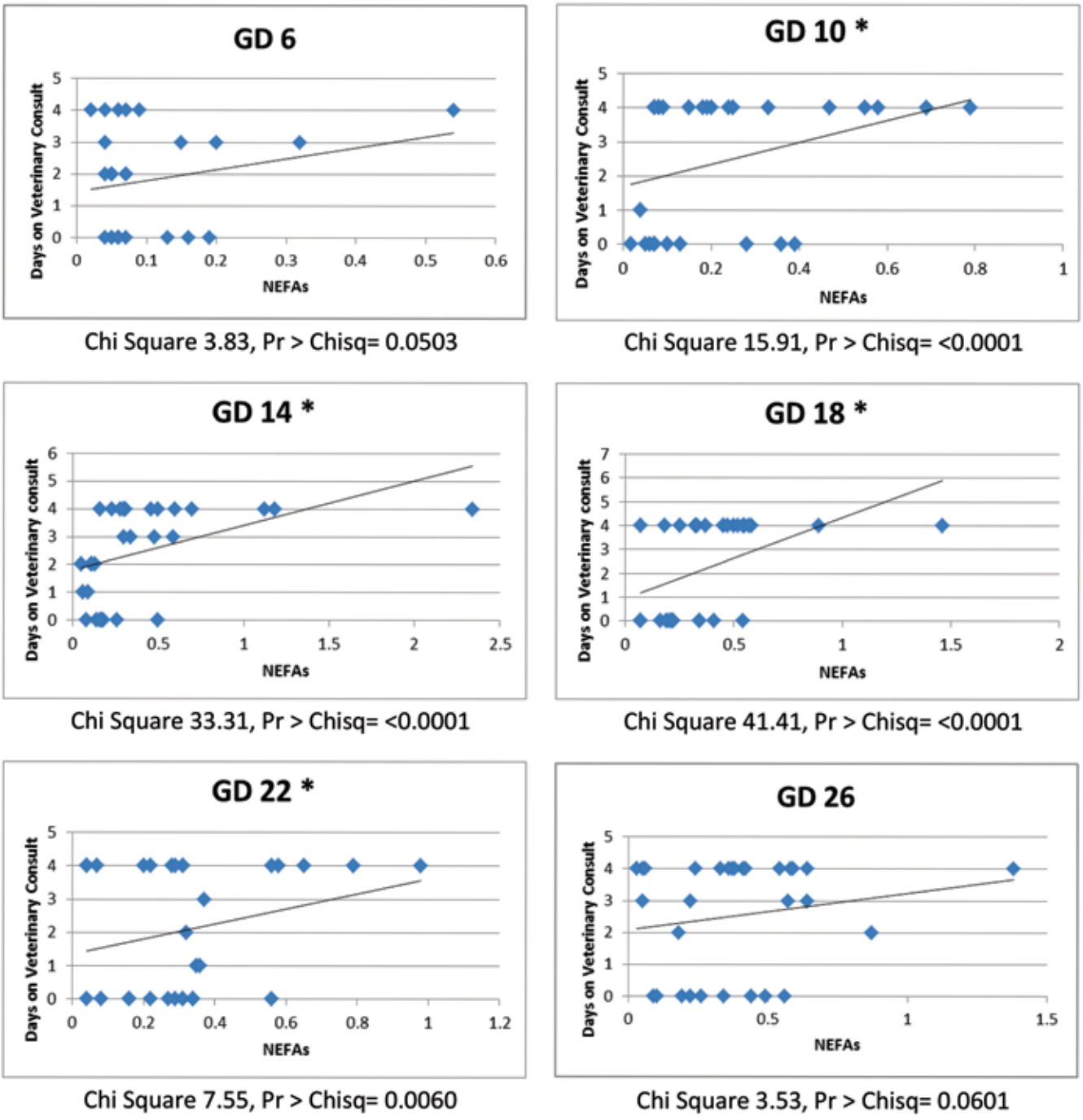

Figure 4. NEFA concentrations are plotted against the number of days on veterinary consultation during the 4 d interval after sample collection. Linear regression modeling results, χ^2 values and Pr > Chisq values are shown, with significant effects in bold (*,Pr > Chisq less than 0.05). During midgestation (GD 10-22) the number of days on veterinary consultation following sample collection correlated to elevated NEFA levels.

Elevated NEFA levels and number of days on veterinary consult, both before and after the collection of the serum sample at multiple points within gestation, is consistent with production medicine in other veterinary species where elevated NEFAs are associated with future clinical problems.[1,2] The finding that more intervals had significant positive correlations between elevated NEFAs and days on consult during the interval after collection (Figure 4) rather than the interval before collection (Figure 3) indicates that NEFAs may predict days on consult and not be simply descriptive.

As described above, NEFA levels and days on veterinary consultation were significantly correlated. Most requests for veterinary consultation due to low food consumption occurred either at the beginning of the study, or after appetite suppression was induced. Later in the study, when food consumption values were normalizing, over half of the animals in both appetite suppressed groups developed injection site complications that required 50% of animals dosed with buprenorphine SR to be placed on veterinary consultation. Since these injection site reactions were another variable in this study that resulted in animals from both H-AS and NH-AS groups to be placed on veterinary consult, the statistically significant association of NEFA levels and veterinary consultation may be confounding the ability to evaluate the effect of diet on energy balance using NEFAs.

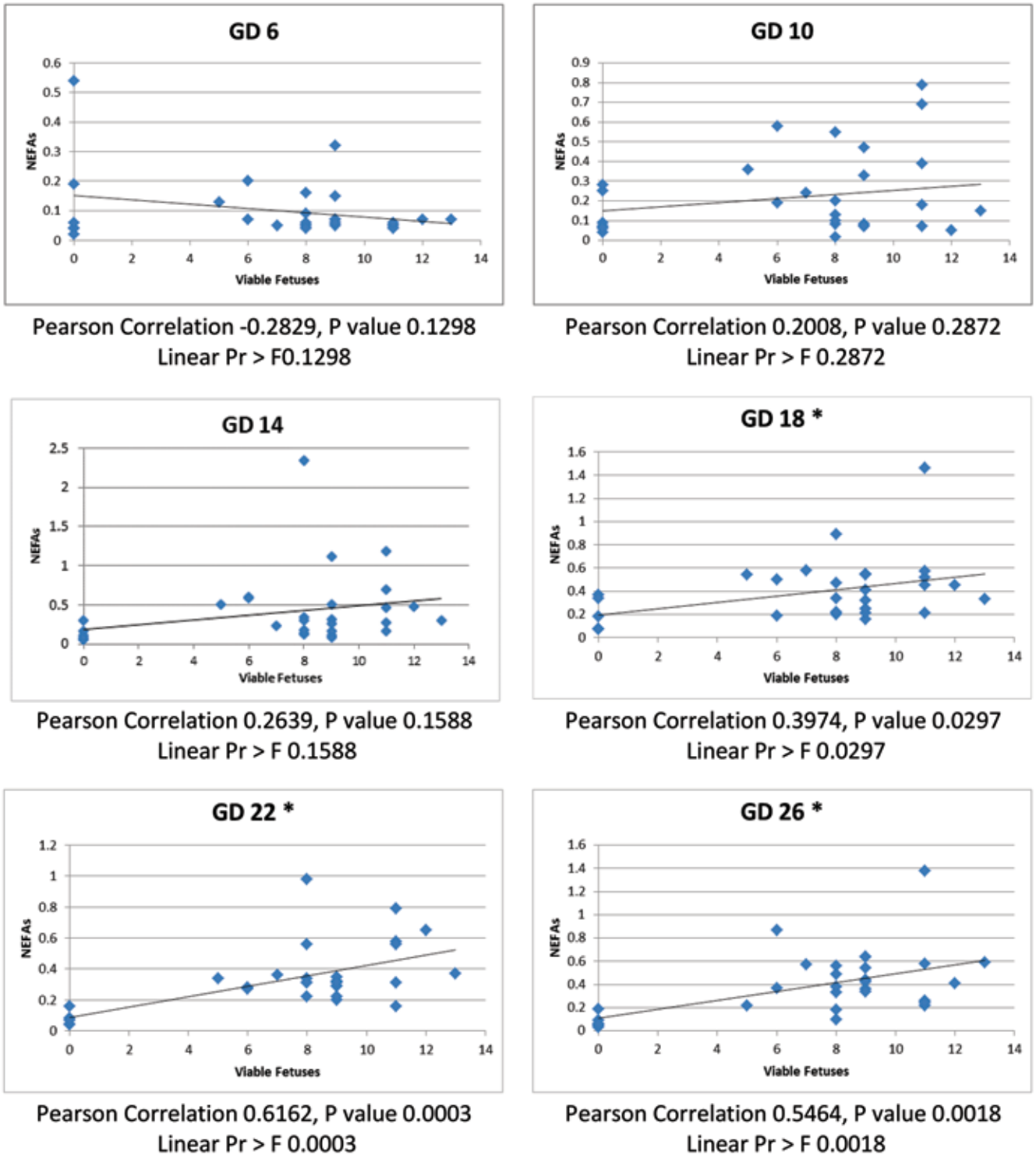

Figure 5. Number of viable fetuses, as determined at necropsy, and NEFA values at each NEFA time point. Pearson correlation and simple linear regression model with type I SS, *P* values and Pr > F values listed, with significant results in bold(*,$P < 0.05$). As gestation progresses, litter size correlates more closely with NEFA levels.

The strong correlation of NEFAs with litter size of viable fetuses (Figure 5) and the total number of implants at the end of gestation is consistent with the biology of other litter bearing species. As the fetuses approach full term and are rapidly growing, they place high energy demands on the dam and cause an increase in energy metabolism that can quickly overwhelm energy intake. As a result, the dam is in a prolonged state of negative energy balance and begins to mobilize adipose tissue to meet the demand.[10,29] The correlation with elevated NEFAs and larger litter size is consistent with the logical assumption that as fetus numbers increase, the energy demand on the dam will also increase. In small ruminants, higher fetal numbers have been shown to increase the risk for pregnancy toxemia, which is the extreme example of late gestational negative energy balance.[21] The correlation identified between NEFA levels and litter size at the end of gestation of this study is consistent with the

Figure 6.

Group	Animal	First study day on consult for anorexia	Last study day on consult for anorexia	Study day treatments administered (novel food items)	First study day on consult for skin lesion	Last study day on consult for skin lesion
NH	271	2	29	2–28		
	272					
	273	7	29	7–28		
	274	15	29	15–28		
	275					
	276	8	28	8–27		
	277	7	29	7–28		
	278	7	29	7–28		
	279	14	29	15–28		
	280	17	29	16–28		
NH-AS	3501	8	22	9–14,16–20		
		27	29	28		
	3502	2	22	3–14	24*	29*
	3503	9	29	9–24		
	3504					
	3505				28	29
	3506				8	29
	3507	3	10	3–8	16	29
		17	21	18–20		
H	1501	7	14	None		
	1502	2	15	None		
	1503	2	15	None		
	1504	24	29	None		
	1505					
	1506	15	29	None		
H-AS	2501	8	22	None	27	29
		27	29	None		
	2502	7	29	None		
	2503	2	15	None	27	29
	2504	8	16	None	27	29
	2505	8	29	None		
	2506	8	29	None		
	2507	8	16	None	28	29

Figure 6. Summary of start and duration of veterinary consult, reason for veterinary consult, and duration of veterinary treatment by animal. *Animal 3502 required topical treatment for skin lesion.

conclusions found in literature evaluating effects of food restrictions on pregnant rabbits on various outcomes.[4,17,20,22] These studies identify the end of gestation as the period when pregnant animals are most affected by food restriction, concluding the energy demand of the fetal growth during this period places pregnant does at greater risk for the metabolic consequences of negative energy balance.

The correlations between NEFA levels and litter size, as well as NEFA levels predicting days on consult suggest other possible applications of NEFAs analysis in rabbits that are already at high risk metabolically. Unlike in dairy cattle where the last 2 wk is the optimal time for evaluating NEFAs, results from this study indicate that litter size begins to have too much of an influence on serum NEFA levels at the end of gestation in rabbits. As a result, NEFAs measured after GD 18 in rabbits would be difficult to interpret for predicting clinical problems during gestation. Since this study did not follow rabbits through parturition and lactation, no conclusions can be made regarding NEFAs at the end of gestation and in the postpartum period. In a research setting with time mated rabbits, a possible clinical application for NEFA levels measurement would be to evaluate shipping stress and as a tool in prestudy selection, due to its predictive nature for days on veterinary consult.

NEFA values were not statistically significant between groups when evaluating diet in this study. Litter size and days on veterinary consultation, factors independent of group, were both significantly correlated with NEFAs, which may have confounded the ability to evaluate diet using NEFAs. However, the relationship of these factors to NEFAs help in our understanding of NEFAs and their potential as a biomarker of energy balance in the pregnant rabbit and help also in directing possible future use of NEFAs as a clinical or diagnostic tool.

Clinical endpoints independent of NEFA levels. The primary effect of feeding hay without retriction was the reduced need for treatment of animals on veterinary consult (Figure 7), which is demonstrated by the reduction in number of days on consult in the hay only group. While food consumption of animals given pelleted basal lab diet fell after administration of buprenorphine-SR, those that had access to hay without restriction did not require additional treatments. Rabbits that have severe anorexia often are offered a variety of produce as well as hay to stimulate appetite, which introduces more diet variables into a study. In a research environment where it is desirable to control as many variables as possible, this study shows that feeding hay to all animals for the entire gestation period reduces the number and variety of diet treatments introduced to the animals on study. In addition, the facts that the animals sustained their appetite for hay and had more consistent fecal output were the drivers in determining that no additional clinical treatments were necessary. This supports the conclusion that the animals were better able to

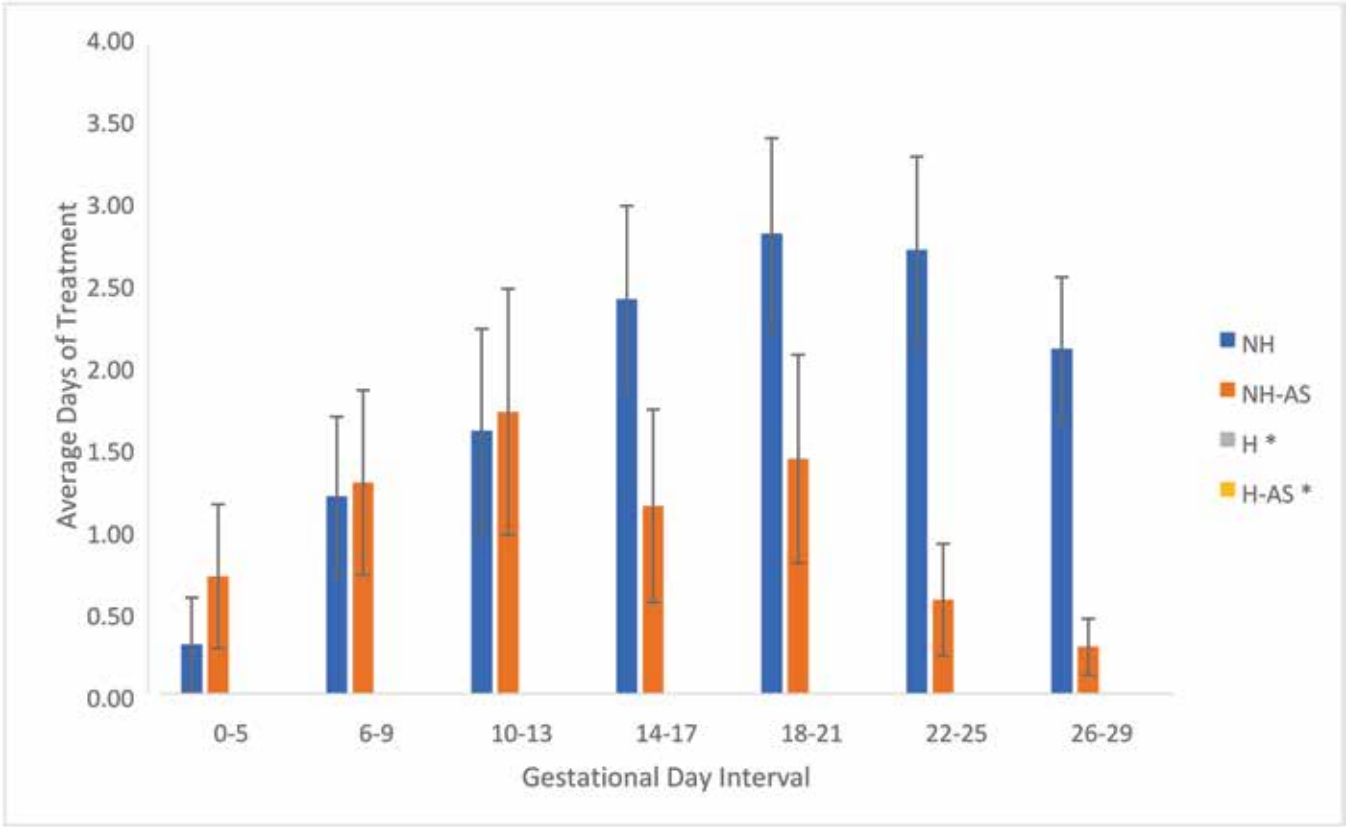

Figure 7. Average number of days requiring veterinary treatment by group. Intervals defined by blood sample collection dates. Animals receiving hay as part of their diet did not require any additional veterinary treatments, Mantel-Haenszel Chi-Square and Fischer exact test performed for significance (*, $P < 0.001$)

clinically manage the appetite-suppressing event. These findings corroborate the literature that states hay is an important part of the diet for rabbits.[14,26,28,31] As a result of these findings and current literature recommendations, certified hay has become a standard portion of the diet of pregnant rabbits within our facility.

Acknowledgments

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