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**Impact of Phosphorus Intake on Beef Heifer
Growth Performance and Conception Rates**

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Abstract

This study aims to examine the effects of phosphorus intake on beef heifer growth performance and conception rates. In Northwest Arkansas, there has been an increase in phosphorus concentrations in soil where livestock manures have been repeatedly applied. Consequently, forages grown on soils high in phosphorus tend to contain high amounts of the mineral itself. This has led many to question whether it is necessary to supplement phosphorus in areas where concentrations may be higher. In this study, crossbred Angus heifers ($n = 72$), approximately 30 days after weaning, were stratified by body weight (average initial weight 251 ± 3.9 kg) and allocated randomly into 8 groups. Groups were assigned randomly to 1 of 2 treatments. Treatments were delivered through either 1) a free-choice-mineral mix that contained no supplemental phosphorus (**CON**), or 2) a free-choice-mineral mix with 4% supplemental phosphorus and identical concentrations of other supplemental minerals (**4PMIN**). Heifers grazed 2.42 ha mixed grass pastures with a history of livestock manure application and were supplemented with soy hulls (0.5% of body weight) daily. Data were analyzed using the mixed procedures of SAS with group as the experimental unit. Total mineral intake through day 112 did not differ ($P = 0.55$) between treatments. On days 84 and 112, any heifers > 273 kg body weight ($n = 58$) had an ultrasound evaluation of their reproductive tract. Reproductive tract score (1, infantile to 5, corpus luteum present) did not differ ($P = 0.65$) due to treatment. Body weights were not different ($P \geq 0.59$) through day 264, 409 ± 6.0 kg and 412 ± 6.0 kg for CON and 4PMIN, respectively. When grazing pastures with a history of livestock manure application, heifers did not need supplemental phosphorus through breeding season.

Keywords: phosphorus, heifers, conception, reproduction

Introduction

Nutrition has a major influence on growth and productivity of livestock. To help an animal achieve its genetic potential, a well-balanced diet of protein, vitamins and minerals is a necessity. While there are different nutrient requirements for each stage of an animal's life, it is well known that phosphorus is a crucial component to the feed ration of any livestock species. In recent decades, Northwest Arkansas has experienced an increase in phosphorus concentrations in the soil where livestock manures have been applied repeatedly. Forages grown on soils high in phosphorus generally also contain high phosphorus. Because of these events, there has been significant debate on whether it is necessary to add phosphorus to feed rations for grazing cattle. This conversation is also fueled by the fact that phosphorus is one of the most expensive minerals to add to rations.

This study aims to examine the effects of phosphorus intake on weanling beef heifer growth performance and conception rates. One group was grazed on pasture with a long history of livestock manure application and fed grain with minimal amounts of phosphorus and given no supplemental phosphorus in a mineral mix, while the other was grazed on similar pasture and fed the same grain and given supplemental phosphorus in their mineral mix.

Literature Review

Phosphorus is a crucial nutrient in animal health and wellbeing. Over three-fourths of this mineral can be found in the body of an animal and is abundant in the bones and teeth of many species (Karn, 2001). Benefits of feeding phosphorus include increased cellular growth, development of musculoskeletal growth and maintenance of body weight. Not only has phosphorus been shown to be vital to animal growth and well-being, but deficient amounts have

been proven to cause reproductive problems. Previous studies have found that beef heifers fed higher levels of phosphorus continue to cycle later in the season over heifers that were fed diets lower in phosphorus (Call et al., 1978).

There has been much discussion on whether it is truly beneficial to add phosphorus to the diets of beef cows. The environmental aspect of this conversation is supported by excess phosphorus in the soil. While price discourages some producers from adding phosphorus to feed rations, studies have shown that well-balanced diets provide shorter anestrus cycles (Ciccioli et al., 2003). Furthermore, nutritionally compromised cows have difficulty maintaining adequate body condition score to exhibit estrous (Hess et al., 2004). As an industry, cattle producers are in need of nutritional programs to increase and maintain fertility in their herds. In order to achieve a highly concentrated period of calving, early onset of puberty in replacement females is crucial (Diskin and Kenny, 2016).

Nutrition affects nearly every aspect of livestock management. More specifically, hormonal influences have been found to correlate with nutrient intake (Velazquez et al., 2008). From a reproductive standpoint, maintenance of hormones is essential for conception to occur (Roberts et al., 2005).

It is also well established that nutrition has a profound impact on fetal growth and development during the last one-half of gestation (Funston et al., 2009). However, there has been limited research as to whether specific minerals influence conception rates in beef cattle. In addition to growth, phosphorus also has an effect on reproductive efficiency and performance. One of the best ways to predict reproductive success in heifers is to measure pelvic area. This procedure is performed using a Rice pelvimeter to measure the height and width of the pelvis. This device can provide valuable data to determine bone size. In addition, pelvic area

measurements also estimate the size calf a heifer can have without significant difficulty (Patterson and Herring, 2017). Primiparous heifers have an increased risk of dystocia due to smaller pelvic openings compared to older cows. By knowing the dimensions of a heifer's pelvis before calving, producers are able to identify which animals may need assistance delivering. From collecting this data between both treatment groups, we will be able to determine if phosphorus plays a role in pelvic area.

Not only does phosphorus promote growth and efficiency, previous studies have suggested that it can affect the reproductive cycle and onset of puberty in heifers. In order to determine onset of puberty, heifers in this study will be subject to monthly ultrasound evaluations. Reproductive tract scores provide a variety of data, including measurements of follicle and ovary size, as well as the dimensions of uterine horns (Holm et al., 2009). In addition to these values, ultra-sounding the reproductive tract can allow technicians to determine the presence of a corpus luteum. This structure is an indication of cyclicity, or that a heifer is coming into estrus.

For decades, pastures in northwest Arkansas have been fertilized with livestock manure from surrounding farms. For this study, soil samples will be taken and measured to determine phosphorus concentrations in the area. Literature has suggested that phosphorus occurs at low concentrations in the majority of soils (Hinsinger, 2000). The main purpose of testing phosphorus in this area is to determine if the repeated application of livestock manure has a significant effect on soil and plant phosphorus concentrations available for cattle. The goal of this study is to determine the growth and reproductive response of primiparous beef heifers when they are fed rations containing varying amounts of phosphorus.

Materials and Methods

Animals and Management

For this experiment, heifers ($n = 72$) were weaned in May 2019 from the University of Arkansas Division of Agriculture Cow Calf Unit in Fayetteville. Approximately 30 days after weaning, heifers were weighed, stratified by body weight, and divided into eight groups. Following this, groups were assigned randomly to one of two dietary treatments. Group A was supplemented with phosphorus and group B was given no supplemental phosphorus. Treatments were delivered through free choice mineral (Table 1). All groups had identical mineral feeders in their pasture and mineral was constantly available, mineral feeders were moved with groups as they rotated pastures every 28 days. Feeders were checked daily and mineral additions were recorded. Every 28 days the mineral remaining in feeders was weighed and mineral disappearance for each group was calculated and expressed on a grams/heifer each day basis. Heifers remained in 8 groups except during the breeding season (days 168 to 223), during this period heifers were kept in 2 groups (1 group/treatment). Heifers remained on their appropriate mineral treatment and mineral intakes were recorded however they were not used in the statistical analyses because of a lack of replication.

Cattle were examined daily to detect morbidity and received antibiotic treatment as required for pinkeye ($n = 6$) and mastitis ($n = 1$). Heifers were given a pinkeye vaccine on day -1 and were treated with a pour on for ectoparasites (Standguard, Elanco, Greenfield, IN) on days -1, 27, 84, 112, and 252. Heifers were treated for endo- and ectoparasites on day 196 (Cydectin Pour-on, Bayer Livestock, Shawnee Mission, KS).

As the breeding season approached, heifers were allotted to treatments in a concurrent research project investigating the use of sexed semen in a short-term fixed-timed artificial

insemination protocol. This project had a $2 \times 2 \times 2$ factorial arrangement of treatments and heifers on this pre-existing nutrition project were stratified across these new experimental treatments to be bred by artificial insemination. In brief, on day 151 half the heifers were administered 5 mL of prostaglandin_{2 α} (**PGF_{2 α}**); 7 days later controlled internal drug release (**CIDR**) intravaginal progesterone inserts and 2 mL gonadotropin release hormone (**GnRH**) were administered to all heifers. After 7 days, all CIDR were removed and all heifers were administered 5 mL PGF_{2 α} . Heifers were inseminated at either 54 or 72 hours after CIDR removal (days 167 and 168) with either sexed or conventional semen. When inseminated heifers also received 2 mL GnRH. On day 179, heifers were exposed to fertile bulls (1 bull/treatment, bulls had passed a breeding soundness exam within 21 days of use), bulls were rotated between groups on day 196. On day 214, a bull was found to be injured and was replaced with a third fertile bull. Bulls were removed on day 224.

Collection Periods and Description

Cattle were grazed on 8 ha mixed bermudagrass and fescue pastures throughout the summer months and supplemented at 0.5% of their body weight with soybean hulls, a low phosphorus feed product. This diet met or exceeded protein and energy requirements. Soil samples were taken in February 2020 and were analyzed at the University of Arkansas Division of Agriculture Marianna Soil Test and Research Laboratory. Soil phosphorus concentrations ranged from 130 to 259 ppm. Forage samples were taken on day 0 and approximately every 28 days thereafter. Samples were collected by walking pastures and taking grab samples from each pasture. Forages were stored in a freezer at -20 °C until analyzed (Table 2). In order to measure concentrations of minerals in the diet, samples were taken from free choice minerals as well the pelleted soybean hulls.

Blood Collection, Processing and Analyses

A subset of heifers (4 per pasture group) were bled every 56 days for analysis of plasma mineral concentrations. Collections were made via jugular venipuncture into vacuum tubes made specifically for trace mineral determinations (Vacuette 456275, Greiner Bio-One GmbH, Kremsmünster, Austria) and plasma was stored at -20 ° C. Following wet ashing, plasma minerals were determined by inductively coupled plasma atomic emission spectroscopy (**ICP-AES**; Arkansas Agricultural Experiment Station Altheimer Laboratory, Fayetteville).

Twenty-eight days after artificial insemination, blood was drawn by jugular venipuncture into vacuum tubes with a clot activator (BD 367985, Franklin Lakes, NJ). Serum was submitted to SEK Genetics (Galesburg, KS) for pregnancy determination by the Biopryn Blood Pregnancy Test (BioTracking, Moscow, ID). Blood samples were again drawn at 30 and 60 days after fertile bulls were placed in the pastures and submitted for pregnancy determination.

Reproductive Tract Scoring and Pelvic Area Measurements

After day 84, any heifers that weighed >273 kg began monthly ultrasound evaluations. Heifers were rectally palpated and evaluated using real-time B-mode ultrasonography to determine uterine horn and ovary size. Reproductive tract scores (**RTS**) were given on a scale of 1 to 5. A score of 1 was given if uterine horns were < 20 mm and no palpable follicles were on the ovaries, while a score of 5 was assigned when the uterine horns were ≥ 30 mm and > 10 mm follicles present as well as a visible corpus luteum (Pence et al., 2000). Heifers weighing >273 kg initially were given a score, while on day 112 a second data collection was completed to obtain data on any heifers that did not meet the weight requirements on day 84 and on those heifers that had an RTS of < 4 on day 84. On day 112, pelvic area measurements were taken using a Rice pelvimeter. This device was used to measure the internal area of the pelvis and area

was determined by multiplying the height by the width of the pelvic opening. Height was measured using the linear distance from the middle of the pubic bone to the bottom of the midsacrum, while width was measured using the linear distance between the ilia (Deutscher, 1987). This data allows producers to detect heifers that could potentially experience dystocia due to small pelvic area.

Statistical Analysis

Mineral intakes were analyzed using the MIXED procedure of SAS (Cary, N.C.). Replicate was a random effect and group was the subject. Treatment, period, and their interaction were fixed effects. Body weights, average daily gains, and reproductive tract scores were analyzed using the MIXED procedure. Replicate was a random effect and group was the subject. Treatment was the fixed effect. Pregnancy data were analyzed using the GLIMMIX procedure. Binary distribution and the compound symmetry covariance structure were specified. Replicate was a random effect and group was the subject. Treatment was the fixed effect.

Results and Discussion

In this study, both groups of heifers were grazed on the same type of pasture and offered soyhull pellets, with treatments being delivered through free choice mineral. Group A was supplemented with phosphorus and group B was given no supplemental phosphorus. In Table 1, amounts were calculated based on a 4 ounce per head per day ratio. The supplemental group received mineral containing 4% phosphorus concentrations, while the control group received a mineral with the same concentrations, only without phosphorus. In Table 3, the supplemental phosphorus group consistently had a higher daily mineral intake compared to the control group ($P = 0.06$). It is important to note that during breeding season (occurring over two periods from

days 166 to 224), bulls and heifers were combined into one replicate per treatment. During this time, the control group experienced a higher mineral intake. This is potentially due to decreasing the number of groups from eight to two.

Forage samples were taken and analyzed to determine neutral detergent fiber (NDF), acid detergent fiber (ADF), crude protein (CP) and ash. Table 2 illustrates a consistent NDF forage value until day 140, where it was highest at 72.67%. Compared to other dietary sources, soyhull pellets had a significantly higher percentage of ADF. Percent ash values varied during the study, with the highest percentage coming from the initial data collection on day 0.

In order to achieve maximum efficiency and performance, growing beef cattle need approximately 0.25% of their diet to consist of phosphorus. Table 5 demonstrates that the phosphorus concentrations of the forages alone were above the minimum requirement for growing heifers. In addition to pasture grass, heifers were given soyhull pellets at 0.5% of their body weight to supplement dietary needs. It can also be noted that the phosphorus concentration of the soyhull pellets combined with forages, heifers were well over their specific requirements. During the winter months, heifers were fed hay and continued to receive soyhull pellets. It is worth noting that the hay consisted of 0.39% phosphorus, a value greater than any concentrations heifers had grazed on earlier in the season. While the concentration of phosphorus in the soyhull pellets were low at a 0.10% value, heifers were only receiving a small portion of their body weight.

Figure 1 and Table 4 show that heifers in both groups were consistent in their average daily gains, with the exception of days 84 to 112 where both groups experienced a decrease in weight gain, but controls gained more than supplemental phosphorus heifers ($P = 0.04$). This overall decrease is most likely due heat stress from summer conditions. During days 141 to 168,

heifers in the supplemental phosphorus group tended to have a greater daily gain ($P = 0.08$) compared to those in the control, however from days 169 to 196, heifers in the control group tended to experience a greater rate of gain compared to the supplemental phosphorus group ($P = 0.07$).

Soil analysis showed the concentrations of phosphorus to range from 130 ppm to 259 ppm. Soils with phosphorus concentrations between 36 to 50 ppm are considered ideal for maintaining optimal forage growth, while those above 50 ppm are considered above optimum. Grasses in this area are excellent consumers of phosphorus. Plant tissue phosphorus will increase if soil concentrations are high in the mineral. Because of this, forages in this area have higher phosphorus concentrations compared to other pastures that do not have a history of livestock manure application.

During this study, blood samples were collected every 56 days to determine plasma mineral concentrations. In this experiment, plasma phosphorus concentrations between groups did not differ ($P = 0.86$). The absence of variation in these values is potentially due to the soil have high concentrations of the mineral itself, however plasma phosphorus is a poor indicator of mineral status. Considering that the majority of phosphorus concentrations occur intracellularly, it is difficult to determine true concentration. Additionally, phosphorus is influenced by other factors such as calcium and protein. Younger cattle will also have higher phosphorus levels due to growth hormone, making it difficult to determine a true status (Qureshi and Deeba, 2019).

On day 84 of this experiment, all heifers weighing > 273 kg were ultra-sounded to determine size of their uterine horns and ovaries, and to check for presence of a corpus luteum (Table 7). There was not a difference ($P = 0.65$) between the groups, with the supplemental phosphorus group having an average score of 3.07 compared to the control group's value of 2.89.

On day 112, there was still no difference ($P = 0.35$) in RTS. From this data, it can be determined that there was little statistical evidence that phosphorus played a role in the growth and development of heifer reproductive tracts.

In addition to ultrasonography, pelvic area measurements were taken on day 112. Between the control and supplemented phosphorus groups, there was little variation ($P = 0.51$) with regards to pelvic measurements.

The control group had 35% of heifers bred to the supplemental phosphorus group's 31% rate ($P = 0.73$). A blood sample to determine whether heifers were bred early in the natural mating season found that 74% of open heifers in the control group versus 52% of the open heifers in the supplemental phosphorus group were bred ($P 0.09$) early in the natural breeding season. After two months, bulls were removed from the groups and breeding season concluded. A blood sample was taken from any heifers open from the last blood draw and was tested again to determine pregnancy status to the bull via the entire natural service period. The results from this collection determined a final 89% and 78% pregnancy rate for the control and supplemental phosphorus groups respectively with $P = 0.19$.

Conclusions and Implications

The main hypothesis of this study was that heifers fed grain rations higher in phosphorus concentrations will have a higher growth and conception rate as a group compared to heifers fed rations that were low in phosphorus. Over the course of this study, there were some variations between groups during periods with regards to average daily gain, reproductive tract scores and total body weight distributions. However, these changes typically came to an equilibrium within the next period. Throughout this study, there were no negative effects of removing phosphorus from the free choice mineral, however, it still remains important to have adequate phosphorus

concentrations in the total diet. Producers in this situation where the land had a history of manure application and forage concentration was 0.35% or greater, could either purchase mineral with or without phosphorus in it.

While the results from this experiment do not completely prove our initial hypothesis, the strongest evidence comes from the pregnancy data. Compared to the control group, the supplemental treatment had an 11% lower end of season pregnancy rate. When looking at other reproductive data, there was little variation between the two treatments. However, in the first attempt at breeding via artificial insemination, heifers in the control group had a higher rate of conception and that trend continued during natural service.

The use of supplemental phosphorus did not seem to hinder the performance of heifers; however, it did not prove to be advantageous. Heifers in the control group performed as well, if not better, in several areas of this study, particularly in regard to pregnancy rates. This data should be used with caution, as this forages phosphorus concentration was high, however, that is not the case with all forages. In this group of primiparous beef heifer that were grazing on pastures with a history of livestock manure application, heifers did not need supplemental phosphorus through breeding season.

The design of this study could be used to further investigate the effects that phosphorus has through calving. While there were little effects shown through growth and performance data, conception rates were slightly higher for those in the control group. Future research could examine the impacts that phosphorus concentrations have through gestation and ultimately parturition.

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Table 1. Composition of free choice minerals

Ingredient	Control	Supplemental P
Calcium, %	20	20
Phosphorus, %	0	4
Salt, %	24 to 26	24 to 26
Magnesium, %	0.2	0.2
Potassium, %	0.1	0.1
Copper, mg/kg	2,500	2,500
Selenium, mg/kg	26	26
Zinc, mg/kg	10,000	10,000
Vitamin A, IU/kg	440,000	440,000
Vitamin D3, IU/kg	22,000	22,000
Vitamin E, IU/kg	22	22

Table 2. Forage composition (dry matter basis)

Date	NDF, %	ADF, %	CP, %	Ash, %
June, day 0	67.23	35.15	14.94	8.41
July, day 27	66.71	30.65	12.31	7.51
August, day 56	69.47	32.81	12.81	7.36
September, day 84	68.23	30.09	14.06	7.67
October, day 112	68.06	31.38	15.38	7.93
November, day 140	72.67	34.23	11.31	6.19
Hay	68.99	31.43	13.13	6.85
Soyhull pellets	67.99	48.83	10.69	5.13

Table 3. Mineral intake (g/day)

Date	Control	Supplemental P	SE	P-value		
				Treatment	Period	Treatment × Period
Days 0 to 27	76.35	91.98	7.34	0.06	< 0.001	0.41
Days 28 to 56	72.3	84.64				
Days 57 to 84	55.89	64.76				
Days 85 to 112	54.95	66.11				
Days 113 to 140	62.19	69.75				
Days 141 to 165	74.54	88.1				
Days 225 to 252	56.52	74.84				
Days 253 to 263	82	123.27				
Overall	66.84	82.93				

¹During 2 periods when with bulls, heifers were housed in 1 replicate/treatment. Consumption was as follows: days 166 to 196 = 105.79 and 60.26 g/day; days 197 to 224 = 80.6 and 76.84 g/day for control and supplemental P, respectfully. These data were not included in the above statistical analysis.

Table 4. Average daily gain

Date	Control	Supplemental P	SE	P-value
Days 0 to 27	0.71	0.70	0.040	0.76
Days 28 to 56	0.62	0.59	0.049	0.65
Days 57 to 84	0.29	0.39	0.042	0.13
Days 84 to 112	0.17	0.01	0.041	0.04
Days 113 to 140	0.50	0.51	0.042	0.84
Days 141 to 168	0.41	0.62	0.069	0.08
Days 169 to 196	1.11	0.95	0.059	0.07
Days 197 to 224	0.65	0.68	0.041	0.58
Days 225 to 252	0.97	1.01	0.067	0.76
Days 253 to 264	0.48	0.58	0.174	0.70
Days 0 to 264	0.60	0.61	0.015	0.70

Table 5. Feed mineral composition

Date	Phosphorus, %	Potassium, %	Calcium, %	Magnesium, %	Sulfur, %	Iron, ppm	Manganese, ppm	Zinc, ppm	Copper, ppm
June, day 0	0.36	2.32	0.39	0.18	0.23	269	95	63	9
July, day 27	0.36	2.20	0.40	0.20	0.23	97	118	56	7
August, day 56	0.37	1.90	0.43	0.20	0.24	175	98	91	12
September, day 84	0.34	2.10	0.44	0.20	0.25	194	67	63	8
October, day 112	0.37	1.91	0.47	0.20	0.26	237	96	206	16
November, day 140	0.28	1.26	0.39	0.15	0.19	171	103	99	8
Hay	0.39	1.52	0.49	0.36	0.25	123	97	94	9
Soyhull pellets	0.10	1.17	0.64	0.23	0.09	393	26	44	7

Table 6. Plasma mineral concentrations

Plasma Mineral	Control	Supplemental P	SE	P-value		
				Treatment	Day	Treatment × Day
Phosphorus, mg/dL	6.35	6.57	0.116	0.19	< 0.001	0.86
Calcium, mg/dL	3.82	3.92	0.071	0.35	< 0.001	0.86
Potassium, mg/dL	15.63	16.28	0.282	0.11	< 0.001	0.76
Sodium, mg/dL	303.48	309.81	1.92	0.03	< 0.001	0.13
Magnesium, mg/dL	1.63	1.64	0.028	0.81	< 0.001	0.42
Sulfur, mg/dL	5.44	5.39	0.070	0.60	< 0.001	0.87
Zinc, mg/L	0.23	0.20	0.010	0.12	< 0.001	0.37
Copper, mg/L	1.06	1.03	0.019	0.26	< 0.001	0.52

Table 7. Reproductive data

Evaluation	Day	Control	Supplemental P	SE	P-value
	112	3.48	3.24	0.18	0.35
Pelvic area, cm ²	112	169	165	4.3	0.51
Pregnancy rate to synchronized breeding, %	196	35	31	10	0.73
Pregnancy rate for early bull bred, %	224	74	52	8.5	0.09
Pregnancy rate at end of breeding season, %	259	89	78	5.5	0.19

Figure 1. Body weights

