EFFECTS OF FEEDING UREA ON REPRODUCTIVE EFFICIENCY IN MICHIGAN DHIA HERDS

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ABSTRACT

EFFECTS OF FEEDING UREA ON REPRODUCTIVE EFFICIENCY
IN MICHIGAN DHIA HERDS

By

Wendell L. Ryder

Data from Michigan Dairy Herd Improvement Association (DHIA) herds was studied to determine if there was a relationship between the feeding of urea and the reproductive efficiency of dairy cattle.

A total of 3157 herd-year observations representing 85,281 calving intervals were calculated from DHIA records for the five-year period 1965-1969. Data concerning feeding practices furnished by the dairymen through a survey form were used to calculate the average amount of urea fed per cow daily for each of the herds. The average total amount of urea fed per cow daily in herds fed urea was 80.6 grams (range 9.0 to 370 grams). The average amount of urea fed per cow daily from various sources was: urea-treated corn silage, 40.0 grams; commercial protein supplements, 32.6 grams; urea addition to dry grain rations, 7.5 grams; and urea treated high moisture corn (grain) 0.6 gram. Adjusted calving interval (ACI), computed as calving interval minus days from calving to first breeding as herd policy, and percent of cows sold for sterility from DHIA records were used as the measures for reproductive efficiency.

The mean ACI for herds fed no urea was 314.4 days compared to 315.7 days for herds fed all levels of urea. ACI for herds fed 1-60 grams urea was 313.4, 61-120 grams 317.8, 121-180 grams 316.5 and over 181 grams 313.7 days. Length of calving interval (days) for the above

groups were: 380.4, 379.9, 379.4, 380.7, 379.8 and 377.8 days respectively. These differences were not significant. Percent of cows sold for sterility in herds fed no urea was 2.15% versus 2.4% for all levels of urea. Though statistically significant, the difference represents only about one more cow in 400 sold for sterility when urea is fed and is of no practical significance and may be unrelated to urea feeding since only 1.71% of cows were sold for sterility in herds fed 181 grams or more of urea.

Correlation analysis of the ACI and total grams urea fed resulted in a simple correlation r = .03, which was non-significant. Partial correlations between each of the sources of urea and ACI were of the order r = .02 to .01 and accounted for only about 15% of the variation in ACI. Similarly, the number of years urea was fed was not related to length of calving interval as indicated by the low correlation (r = .01) for year by urea interaction and adjusted calving interval. From the statistical analysis of the data it was concluded that neither the level of urea fed nor the number of years urea was fed affected reproductive efficiency of dairy cattle in Michigan DHIA herds. Average calving interval was 5 to 6 days longer for the years 1967 and 1969 than for the other three years studied whether or not urea was fed. Reasons for the lower reproductive efficiency some years than others were not revealed by this study.

These findings agree with the results of research reported in the literature where cow numbers were much smaller.

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INTRODUCTION

The importance of nutrition in the production performance of dairy cattle has long been the subject of exhaustive study and research. The relationship of nutrition and reproductive efficiency in dairy breeds has also been under some research scrutiny for many years.

Reproductive efficiency has become increasingly more important as good nutrition and management factors have consistently raised the average production per cow year after year.

Though this investigation is concerned primarily with only one nutritional factor and its relationship to reproductive efficiency, the effects of several nutrients involved in reproduction are briefly reviewed. Because infertility may be the result of any one of many, or a combination of factors considerable difficulty has been encountered by investigators in assessing the effect of diet on reproduction. The relationship of dietary factors such as energy, protein, minerals and vitamins to reproductive performance of dairy cattle has been investigated by some workers. Several of these studies will be outlined in the literature review.

In the investigation reported herein the use of non-protein-nitrogen primarily in dairy cattle diets has been studied to determine its effect on reproduction efficiency of cattle fed and managed under farm conditions.

Since World War II the tonnage of urea used by Dairy and Beef producers in the United States has doubled and quadrupled from 60,000 tons in 1956 to over 200,000 tons in 1965. The acceptance and use of urea as a replacement for the higher cost plant protein continues at an accelerated pace.

The use of urea as an economical and efficient source of protein for dairy cattle in Michigan has become widespread since 1964. It is currently being added to corn silage, high moisture corn, dry grain mixes and by feed manufacturers to protein supplements.

In the past two or three years increasing numbers of reports from the field indicate that the use of urea is being blamed for difficulties in dairy cattle, ranging from toxicity to poor reproductive performance. Reproductive failures and poor conception as might be related to urea feeding seem to be uppermost in the minds of dairy producers, field extension personnel and practicing veterinarians.

There has been much research work done on the use of urea in dairy cattle diets over the past 15 years. To date this research has not indicated any affect on reproduction *per se* from the use of urea. It should be pointed out, however, that very little of the research work completed involving urea, was designed to test its effect on reproduction, rather the investigators measured feed intake, growth rate, or milk production.

The persistency of field reports that feeding urea and poor reproduction go hand in hand warranted the further investigation by this researcher to ascertain whether these reports are valid or whether the poor reproduction was caused by other management procedures.

The object of this investigation was to determine whether the urea fed to dairy cattle significantly altered calving intervals used to measure reproductive efficiency. Should urea prove to affect calving interval a secondary objective was to evaluate the loss from lengthened calving interval in light of the savings in feed costs resulting from feeding urea.

REVIEW OF LITERATURE

Nutritional Factors of Importance in Reproductive Efficiency

Research workers over the years have postulated many theories as to the cause of sterility, failure of estrous, cessation of ovulation, fetal deaths, abortions, dead or weak offspring at birth and a host of other unexplained reproductive failures. The purpose of this research effort was concerned primarily with the effect of only one nutritional factor, urea, upon reproduction in dairy cattle.

However, since several nutritional factors play interacting roles in total reproductive efficiency, the research work concerning the role of individual nutrients as related to reproductive success or failure is reviewed. The nutritional factors which are considered are level of energy, protein, vitamins, minerals and the non-protein nitrogen compound, urea.

Classic Pioneer Study

In the early 1900's Wisconsin workers began to be concerned about reports from various parts of that state regarding serious reproductive failures in dairy herds. Upon closer inspection it was noted that in certain sections of Wisconsin the incidence of dead or weak calves at birth was abnormally high, while in other areas this was either not true at all or was much less serious. As the situation became more serious Agricultural Experiment Station investigators began to consider what differences existed in diets of dairy cattle in different areas of that state. It was found after considerable investigative effort that areas where dairy rations consisted largely of the wheat plant a substantial number of calves were born weak or dead. In other areas where corn was the principal grain in dairy rations no such difficulties were encountered. In fact, in these areas newborn calves were exceptionally strong and vigorous.

In still other areas of Wisconsin where dairy rations were formulated largely from the oat plant the situation of calf mortality and vigor at birth was in between the extremes described above.

Hart et al. (38) reported in the Wisconsin Agricultural Experiment Station Bulletin in 1911 of work started in 1907 concerning this phenomenon. Where workers initiated experiments with dairy heifers in which four equal groups of animals were fed rations balanced from the 1) wheat plant, 2) corn plant, 3) oat plant, 4) a combination of all three grains. These experiments were carried through several reproductive cycles. In this early classical nutrition study, Hart et al. (38) discovered that dairy cattle fed entirely on a ration balanced from the wheat plant had severe reproductive disorders with calves being born dead, or so weak they died shortly after birth. Estrus cycle was interferred with and some animals became blind. The cattle on rations balanced from the corn plant had normal reproduction and normal healthy offspring. The cattle on rations balanced from the oat plant had some reproductive disorders and some weakness in calves at birth. However, the cattle on rations balanced with a combination of the three grains had normal reproduction and normal offspring without the benefit of the host of nutritional factors yet to be discovered. These early workers concluded that the wheat plant ration was incomplete in nutrition due to inadequate mineral content, and to the presence of a toxic factor in the wheat grain. After the discovery of certain vitamins important in nutrition, the Wisconsin workers conducted additional research in this same area and placed a new interpretation on the 1911 study. They concluded that the primary reason for the superior response to corn over wheat was because of its higher carotene (pro-vitamin A) content.

Level of Energy

In farm animals undernutrition delays the onset of puberty and leads to impaired fertility in mature animals. Steensberg (96) and Allen (1) both reported that heat symptoms occur at later ages in under-fed heifers than in heifers fed adequate diets. This fact was clearly illustrated by Reid et al. (90) in experiments with calves and heifers fed normal (Morrison's standards), above-normal (140% of normal) and sub-normal rations (65% of normal). On an average, the animals experienced their first heat period when they were 11.3, 9.4 and 17.3 months of age, respectively. The underfed animals had the poorest conception rate, underdeveloped udders and in many cases, the animals gave birth to dead calves. In mature cows, Tuff (99) found that underfeeding impaired normal estrous symptoms and reduced fertility.

In sheep, several investigators have demonstrated that insufficient nutrients may impair fertility. Bellows et al. (10) showed an increased ovulation rate in ewes by nutritional flushing (increasing the level of energy just before breeding) and suggested that the responsible endocrine mechanism is subject to modification by the level of feeding. El-Sheikl et al. (25) fed 80 mature ewes (mixture of Shopshire, Hampshire, Oxford) two nutrient levels in a two-year study in which reproductive capacity was measured. One group of ewes received daily 2 lb of a grain supplement mixture plus alfalfa-brome grass hay while the other group received only hay or pasture. Results of this study clearly demonstrated that the ewes on the higher level of feeding had sugnificantly higher ovulation rates, larger follicles, and a higher number of follicles 2 mm or more in diameter.

Kirkpatrick et al. (58) reported that energy levels did effect ovulation rate with ovulation rate being greater at the higher energy level tested. Different patterns of pituitary gonadotropin production or release between level groups were indicated. Residual pituitary FSH activities tended to be lower early in the estrous cycle, higher at mid cycle and lower late in the cycle in gilts fed glucose than in those fed a basal ration. Residual pituitary LH activities tended to be lower early in the cycle and higher at mid cycle in the basal fed gilts than in those fed glucose.

Howland et al. (51) in 1966 reported work on two nutritional levels in ewes and effects on pituitary and ovarian function. This long-time study involved a total of 96 mature ewes, half of which were fed a high energy ration (grain) while the other groups were fed just hay (low energy). The animals were studied at four stages of the estrous cycle (days 1, 4, 10 and 16). Ewes fed grain had heavier adrenal and pituitary gland weights and number of ovulated eggs was higher. Follicular fluid weights and number of large follicles were greater in grain fed animals at all stages. This work suggested that persistently elevated plasma glucose levels, via hypothalmic stimulation, lead to greater gonadotropin production and subsequent greater ovarian activity.

Dunn, et al. (110) showed that level of energy intake can markedly alter reproductive performance in 2-year old beef heifers nursing their first calves. Pregnancy rate 120 days after calving was directly related to the post-calving energy level. Eighty-seven percent of the cows fed the higher energy (48.2 megcal. digestible energy) level after calving were pregnant compared with 72 percent of those fed the moderate level (27.3 megcal.) and 64 percent of those fed the low energy (14.2 megcal.) level (0.01 P 0.05). The onset of estrus was delayed in cows fed a low energy level before calving.

Protein and Reproduction

Adequate protein intake in farm animals as well as laboratory animals are important for proper development and function of reproductive organs as well as for prenatal nutrient needs and fetal requirements.

Protein deficiency in farm animals is generally believed to give rise to reproductive disturbances. Underdevelopment of the ovary and uterus are common findings in heifers fed diets inadequate in protein. This fact was verified by Reid et al. (90) with their study of the effect of sub-normal rations on growth and development of heifers and calves. The importance of adequate protein intake for the development and function of the female reproductive organs is well known from experiments with laboratory animals as described by Leathem (60) in "Sex and Internal Secretions." In swine, Davidson (20) reported that protein deficiency resulted in impaired development and function of the reproductive organs.

Palmer et al. (80) studied the effect of rations deficient in protein and phosphorus on ovulation, estrus and reproduction in dairy heifers. In this early study eleven grade Holstein heifers were fed protein and phosphorus deficient diets for from 24 to 59 months duration for different animals. These ration restrictions imposed conditions analogous to similar deficiencies in animals reared largely on prairie grass hay in phosphorus deficient areas. In this experiment the combination of inadequate protein and low phosphorus resulted in delayed sexual maturity and repressed normal estrous but did not interfere with regularity of ovulation or conception. The author suggested that the effects on reproduction in this experiment were comparable to natural occurring results in areas where protein and phosphorus were both deficient.

Numerous studies in the past have suggested that a relationship exists between protein metabolism and vitamin A utilization. If such a relationship existed, certainly reproduction performance could be affected particularly, if this vitamin A-protein interaction resulted in reduced vitamin A utilization. Rechcigl et al. (89), in experiments with male weanling rats (Holzman strain), studied the effect of dietary protein and vitamin A utilization. A large does of vitamin A acetate in oil solution was given orally to all rats in order to increase their vitamin A reserves. The effect of the level of protein and quality of dietary protein were studied to determine their effect on vitamin A utilization. Rats receiving no protein retained less vitamin A than groups receiving protein, as measured by liver storage levels of vitamin A. In general, vitamin A stored in the liver was lowered as the protein level in the diet was increased. The utilization of vitamin A was also affected by protein quality. In rats ingesting an 18% casein diet, less vitamin A was observed in the liver and more in the kidneys than in the slower growing animals fed 18% of either gluten or zein. Amino acid supplementation of gluten and zein resulted in an improved growth rate, increased depletion of hepatic vitamin A, and greater total utilization of the vitamin.

Certainly rats and dairy cattle are vastly different in their metabolism of protein and vitamin A, but these experiments give insight into the general relationships of these nutrients. Should vitamin A status in dairy cattle be measurably affected by the interaction of dietary nutrients, reproductive efficiency might well be affected.

Pregnancy, in terms of nutrition, represents a physiological condition in which all nutritive requirements of the mother become greatly intensified particularly during late stages of gestation. This applies not merely to requirements concerning energy and protein, and macroelements such as calcium and phosphorus but also to requirements for trace elements. Should supplies of nutrients be insufficient to provide the requirements of the maternal organism and requirement for the development of the products of conception; then the fetus will have first priority for the limiting nutrients. This is probably because the distribution of nutrients to organs and tissues in animals is related to metabolic rate which is much higher in the fetus than dam.

Vitamins and Reproduction

Vitamin E:

The author well remembers the great amount of publicity attendant to the discovery and manufacture of certain vitamins in the late 1930's that were claimed to be the cure all for sterility in dairy cattle. Vitamin E (alphatocopherol) and wheat germ oil, a rich source of the vitamin, were sold throughout the country as sensational cures for breeding difficulties in cattle. The author spent much hard earned money on wheat germ oil in an attempt to solve a breeding problem in a vocational agriculture project involving a dairy heifer, but to no avail.

Before vitamin E was recognized, Evans et al. (27) discovered that rats were sterile when reared on a dietary regime consisting of "purified" protein, fat and carbohydrates to which were added a salt mixture and certain growth vitamins.

In an earlier investigation Evans et al. (27) had witnessed a comparatively studden restoration of fertility to rats of proven sterility by the administration of fresh green leaves of lettuce while controls continued sterile. It was concluded that certain natural foodstuffs contained a substance "X" which prevented sterility in rats. In later experiments when wheat germ was used as a source of vitamin B, fertility in rats was restored. It was concluded that this restoration of fertility was not due to more vitamin B but that wheat germ was rich in factor X.

With the discovery that the factor X in lettuce leaves and wheat germ oil was in fact vitamin E, additional investigations were conducted to ascertain the role of this vitamin in reproduction in several species. The importance of vitamin E in reproduction as determined earlier by Evans and Bishop (27) in rats, was later confirmed in both rats and mice by Beard (7), Evans (26) and Mason (63).

In early experiments with Red Danish Cattle Vogt-Moller et al. (104) were quite successful in the treatment of sterility. These investigators, by injection of 10-20 cc of wheat germ oil, were able to bring about pregnancy in 10 of 12 experimental cattle that reputedly were sterile. Gullickson et al. (34) however, in lengthy experiments at Minnesota with 15 cows on E deficient rations for over four generations could not duplicate the results of Vogt-Moller. In the Minnesota work 15 calves of mixed breeding, consisting of nine females and six males, were fed E deficient rations to determine if successful reproduction could be accomplished in the absence of the vitamin. The positive controls in this experiment were fed according to the same plan except that a supplement rich in vitamin E was added to their diet. The vitamin E deficient cattle, bulls and heifers alike, were no different in their sexual development or behavior than were the cattle on rations abundantly supplied with vitamin E. Breeding and calving records were kept on all animals which showed that the reproductive ability of the cattle was not adversely affected by feeding the vitamin E poor ration continuously through three generations.

Vitamin A:

The work of Wisconsin investigators (38) with reproduction in cattle on rations balanced from the corn, wheat and oat plant was discussed earlier. The importance of this earlier work became clear with the discovery of vitamin A. Hart (39) and co-workers at Wisconsin, with the advent of vitamin A, carried out a new research project in which they used a ration balanced with wheat but to which they added bone meal (calcium phosphate) salt and a vitamin A source, raw cod liver oil. When this fortified, wheat-plant based ration was fed to dairy cattle, reproduction and offspring were completely normal.

Vitamin A deficiency in cattle results in blindness (especially night blindness), edema, reproductive disturbances, and as a secondary effect, lower milk production. The dead and weak calves as reported in the 1911 Wisconsin study are characteristic of vitamin A deficiency. Feeding of animals on diets grossly deficient in vitamin A may lead to irregularities in estrus and to diminished fertility through suppression of ovulation or failure of implantation.

McCollum et al. (64) reported suppression of ovulation in animals on vitamin A deficient diets. Warkany (108) in experiments in 1945 with cattle and sheep showed that estrus and impregnation are not greatly affected by a vitamin A deficiency but gestation was disturbed.

On the other hand, Lutwak-Mann (62) reported in 1958 that cattle on a diet deficient in vitamin A showed estrus cycle disturbances. Support for the Lutwak-Mann work comes from a recent (1960) investigation by Highnett (47) who found both estrus cycle disturbances and infertility in cows fed a diet deficient in vitamin A.

Vitamin A deficiency is probably not of great practical importance since it is easily avoided under most farm conditions where good quality alfalfa hay, green chop or corn silage are available, and vitamin A supplements are economical and readily available.

Vitamin D:

Wallis (106) observed reproductive effects of vitamin D deficiency in experiments with mature dairy cattle. In these studies the cows showed estrus during D deficiency and the calves showed poor bone development. Wallis stated however, that it was not clear whether the condition of lack of estrus in D deficient cows should be attributed to the lack of D *per se*, or to the decline in general health and vigor of the animals or perhaps some other factor entirely. Wallis et al. (107) in an earlier study with calves had shown the relationship between vitamin D and the calcium and phosphorus retention when they demonstrated a 14-fold increase in calcium retention and an 11-fold increase in phosphorus retention by vitamin D therapy. It has not been established that uncomplicated deficiency of vitamin D has any effect upon reproduction.

Minerals and Reproduction

During this century, considerable evidence has accumulated indicating that mineral deficiencies may have marked effects upon reproduction. In most cases mineral deficiencies in rations occurred when the ration ingredients were grown on mineral deficient soils. Over the years the effects of calcium and phosphorus deficiencies on reproduction in dairy cattle has received more attention than the other minerals.

Macro-Minerals

Calcium:

In a series of classic studies starting in 1921 Hart et al. (40) directed their efforts to determining the dietary factors influencing calcium assimilation in dairy cattle. The requirement for calcium and phosphorus supplementation with various types of grasses was determined by Hart et al. (41) in experiments reported in 1923. Further research with calcium by Hart et al. (42) showed the influence of sunlight upon calcium equilibrium in cows. This work was reported in 1926 and paved the way to an understanding of the relationship between vitamin D and calcium and phosphorus assimilation. These early workers did not report much regarding reproduction except to indicate that the cows calved normally even when fed calcium deficient rations. Hart et al. (44), in experiments reported in 1929, indicated that ultraviolet light had no apparent effect upon calcium and phosphorus metabolism in dairy cattle. Later experiments conducted by Hart et al. (45) showed that irradiated yeast (a yeast potent in vitamin D) had no positive influence upon calcium assimilation of milking cows fed liberal amounts of a good ration.

Meigs et al. (68) fed low calcium rations to dairy cattle and reported that reproductive difficulties were encountered. Whether these difficulties were due to calcium or phosphorus or the ratio of the two was not understood at that time. Eckles et al. (23) in 1926 reported that cows on severe deficiencies of calcium and phosphorus did not become pregnant, estrus was delayed and ovaries were atrophied. Eckles (23) concluded from this work that cattle could not successfully carry a fetus on rations containing less than 0.32% calcium. Calcium to phosphorus ratios and their interaction were not understood at this time, thus their conclusion was subject to more carefully controlled research.

In 1932 Fitch et al. (30) completed a more exhaustive study on level of calcium and its influence on reproductive efficiency. Three groups of seven cows each were fed one of three levels of calcium, 0.18, 0.32 and 0.64% of the dry matter. The low calcium level group was kept on the program for three years. All other nutrients were supplied in sufficient quantities. This experiment clearly demonstrated that low calcium, when other food elements were in good supply, did not result in lessened breeding efficiency. Cattle on all three levels of calcium had equal reproductive efficiency.

Becker et al. (8) in Florida worked with calcium deficient roughages for dairy cattle and concluded that reproduction represented a much smaller drain on calcium and phosphorus than lactation. According to Becker et al. (8) cows at the Gainesville, Florida Agricultural Experiment Station herd prior to 1933 had been fed unsupplemented rations extremely low in calcium. Yearly herd records indicated that numerous animals had incurred broken bones in the period prior to 1933. The records of this Station did not indicate that reproduction, conception or other breeding difficulties were abnormal prior to publication of the 1933 bulletin.

In later work, Palmer et al. (79) conducted experiments with calcium levels of 0.12% of the dry matter in rations otherwise nutritionally complete. This low level of calcium did not appear to alter breeding efficiency.

The importance of calcium, its interrelationships with vitamin D, phosphorus and other dietary factors is still the subject of continuing research. There is conflicting experimental evidence regarding the role of calcium in the reproduction process, but most of the experimental evidence indicates that calcium level *per se* has little or no effect on reproductive efficiency.

Phosphorus:

In farm animals, reproductive failure due to phosphorus deficiency has been noted mainly in range cattle, as first described by Tuff (99) in Norway and Theiler et al. (97) in South Africa. The South African workers conducted an extensive breeding experiment with cattle on phosphorus deficient ranges. This work showed that cattle not receiving bone meal had a strikingly subnormal calf crop (51 calves born per 100 cows) when compared to the calf crop (80 calves born per 100 cows) of cattle receiving supplemental bone meal. An irregular breeding pattern was also noted in the phosphorus deficient group. This work did not test uncomplicated phosphorus deficiencies since other nutritional imbalances may have been involved.

Eckles et al. (24) in 1935 conducted experiments with mature dairy cows where an uncomplicated phosphorus deficiency was maintained for two or three years. These workers noted no disturbances in the estrous cycle, however, breeding efficiency did seem to be reduced. The conclusion of these workers was that the disturbances in estrous and reduced calf crop reported to occur under natural conditions in phosphorus deficient areas, was probably due to the nutritive deficiencies that accompanied the lack of phosphorus, or to a combination of deficiencies and was not necessarily exclusively a phosphorus deficiency.

Huffman et al. (54) did some classic work in Michigan, published in 1933, on the phosphorus requirements of dairy cattle when alfalfa hay furnished the principal source of protein. Experimental rations contained less than 0.2 percent phosphorus. These investigators worked out the phosphorus requirements for growth, maintenance, reproduction and various levels of milk production for dairy cattle. Reproductive disturbances were noted by Huffman et al. (54) but they made no definite conclusions because of the limited number of animals in the experiment. An extreme lack of appetite was evidenced in some of the cows on low phosphorus which might account for the reproductive disturbances noted since overall nutrition and particularly energy intake was thus affected.

Palmer et al. (80) reported that in heifers, sexual maturity was delayed markedly and signs of estrus were repressed by phosphorus and protein deficiencies but neither regularity of ovulation, or the ease of conception were affected. Growth and development of these heifers were markedly depressed. It was concluded, in view of an earlier study by Eckles and Palmer et al. (24) with uncomplicated phosphorus deficiency, that in this experiment the reproductive disturbances were due primarily to a protein and not a phosphorus deficiency.

From a practical standpoint, phosphorus deficiencies in rations due to soil shortages can easily be corrected by feeding a phosphorus-rich ingredient. For more permanent results fertilization of the deficient soils with phosphorus is easily accomplished.

Micro Minerals

Manganese:

Orent and McCollum (77) were the first to demonstrate that pre-natal manganese deficiency in rats might lead to a reduced viability or to locomotor disorders. Male rats showed some testicular abnormalities but female rats had normal estrous cycles. Boyer et al. (15) in later studies with rats on manganese deficient diets demonstrated that in female rats the estrous cycle was irregular or absent and there was a marked delay in the opening of the vaginal orifice. In males manganese deficiency caused testicular degeneration and complete sterility due to lack of spermatozoa production. In light of this study the greatly reduced reproductive effects shown by Orent et al. (77) in their earlier study might be explained by the fact that their experimental diet might not have been manganese free.

Everson et al. (28) worked with manganese deficient diets for guinea pigs. These workers found that such diets caused a reduction in litter size and a high percentage of the young were born dead or prematurely. Plumlee et al. (85) in 1956 reported that gilts on a low manganese ration showed excess fatness, reduced body length and height and shorter legs. These symptoms suggest that general skeletal development was retarded and that fat deposition was increased. The gilts also had irregular estrus cycles, resorption of fetuses, birth of small weak pigs and poor udder development as well as an almost complete absence of milk secretion.

Hurley et al. (55) in 1963 studies with manganese deficient pregnant female rats determined that manganese supplements added to the diet for a 24 hour period on the 14th day of pregnancy, solved most of the reproductive difficulties encountered in a severe manganese deficiency. On unsupplemented diets with pregnant females, only 11% of the fetuses survived pregnancy and of those born 81% were ataxic. When the manganese supplements were added on day 16 and 18 of pregnancy the number of fetal death and percent of those born alive that were ataxic increased.

In dairy cattle Bentley et al. (11) reported that on low manganese rations dairy cows exhibited delayed estrus and conception, and Hignett (47) produced highly suggestive but not conclusive evidence that the dietary manganese level was important for fertility in heifers.

The full significance of manganese requirements and their relationship to reproductive efficiency and overall health in animals has never been thoroughly investigated. Additional research will be needed to fully understand the role of manganese in the nutrition of animals.

Zinc:

The important relationships of zinc in animal nutrition and its effect if any upon reproduction are still not clearly understood. It has been demonstrated by many workers that zinc is required as a part of several metalloenzyme systems. Miller et al. (70) of the University of Georgia reported work concerning the effects of zinc deficiency, and restricted feeding on wound healing in Holstein calves. Wounds were surgically imposed in three groups of calves which received: 1) a nutritionally complete control diet; 2) a restricted amount of the control diet; and 3) a zinc deficient diet. Results showed a normal, rapid rate of healing on the control diet. In calves fed the restricted control diet healing appeared normal but the generation of new skin was at a much slower rate. In the calves on the zinc deficient diet growth rate of new skin was also retarded but in addition, large areas around the wounds became parakeratotic. Reproductive performance in Holstein bulls that had been severely zinc deficient from 8 to 21 weeks was reported by Pitts et al. (84) in 1966. The deficiency *per se* caused a reduction in testicular size at 21 weeks but the difference disappeared by 64 weeks of age. No definite effects of zinc deficiency were noted in the volume of semen produced, spermatozoa concentration, total number of spermatozoa produced or motility rate of sperm. These results are in contrast to the work with rats by Miller et al. (69) when zinc deficiency caused severe atrophy of the testes, but no reversal effects on the germinal epithelium were noted when zinc was later reinstated in the diet. Voelker et al. (103) studied zinc supplementation in dairy cattle rations where corn silage was the only forage fed and they noted significant increases in milk production with cows on the zinc-supplemented rations. Protein, fat and minerals in the milk were slightly higher on the zinc supplemented rations.

The effects of zinc upon reproduction in the female bovine, have not been established. Since zinc is required in certain metalloenzyme systems, a zinc deficiency would probably affect reproduction in some manner, if only from a growth retardation standpoint.

Ordinarily zinc is in plentiful supply in most dairy cattle rations so is not usually supplemented.

The relationship of other trace elements to reproductive efficiency in dairy cattle rations has not been adequately studied. Other than iodine, it is doubtful that routine supplementation of any trace elements is necessary.

Urea-Nutrition and Reproduction Situation

History of Urea in Ruminant Rations

Rutherford discovered nitrogen in 1772 and named it "aer moligus" or noxious air probably due to the inability of the newly discovered gas to support life. Chaptal in 1790 gave it the name nitrogen to express the characteristic exclusive property of the gas to form the radical of nitric acid.

The first metabolic experiments with nitrogen were performed by Lavoisier in 1790, only 18 years after the discovery of nitrogen. These findings showed that gaseous nitrogen did not play a role in the nitrogen metabolism of mammals but this original finding was to be disputed for many years.

Magendie in 1816 utilized the chemical techniques available to him to show that foods differed in nitrogen content. He demonstrated with dogs that nitrogenous components of the diet were necessary for life.

Urea, a nitrogen containing compound, was first identified in urine by Rouelle in 1773. By 1824 Promst made the first accurate analysis of urea and determined its correct empirical formula. Wohler in 1825 first demonstrated the synthesis of urea from inorganic substances. For many years physiologists and biochemists labored to extend their knowledge of the metabolic pathways involving nitrogen. The unique role of the ruminant microorganisms in the nutrition of the host animal was recognized by both Zuntz (109) and Hagemann (35) in experiments testing the nutritive value of non-protein nitrogenous compounds with ruminants in 1891.

In the next 35 years the ability of these rumen organisms to utilize non-protein nitrogen was explored by many researchers and their theories were vigorously discussed and contested.

Morgan and his associates (74) in a series of experiments with sheep dating from 1907 to 1924 showed that 30% to 40% of the protein in their rations could be replaced with urea. How the non-protein nitrogen provided in the ration was actually used by the ruminants was left for later workers to determine.

Voltz (105) was one of the first to test the theory that non-protein nitrogen could be used by rumen microorganisms to form protein useful to the host animal. He reported in 1907 that he obtained growth with lambs on a low protein, semi-purified diet made up of starch, alkali washed straw, inorganic salts and urea.

When Armsby (2) of the U.S.D.A. published a comprehensive review of the work on the utilization of non-protein nitrogen by ruminants and non-ruminants, the role of rumen microorganisms in converting non-protein nitrogen to protein, their digestion and subsequent use by the host animal as protein for growth and milk was more clearly understood.

The development of the Haber-Bosch synthetic ammonia process with the production of large amounts of by-product carbon dioxide stimulated the successful development of a process to synthesize urea from ammonia and carbon dioxide.

Honcamp et al. (50) were among the first investigators to show that urea could be used in practical rations for milk cows.

In the United States few experiments with urea were conducted in the twenties and thirties. It was known but widely disputed that urea and other non-protein nitrogen sources could be used to replace a portion of the protein in ruminant rations. Hart et al. (37) at Wisconsin conducted and published the results of the first intensive research in the United States on the use of urea and ammonium bicarbonate in ruminants. These workers concluded that ruminants could use simple nitrogen compounds through the action of rumen microorganisms. In Great Britain, Bartlett and Cotton (5) experimented with urea in dairy rations. Their work showed that dairy heifers could effectively use urea. Owen et al. (78) confirmed this with producing dairy cows.

Through the thirties and the forties the role of urea and other non-protein substances and their relationships to nutrition and protein replacement in diets of both monogastrics and ruminants became quite well established. The ability of rumen microorganisms to utilize non-protein nitrogen from urea is credited with averting a protein shortage for the United States during World War II. The tonnage of urea used for replacement protein in ruminant rations has steadily increased and exceeded over 200,000 tons in 1967. Its use continues to expand but speculation about its effect on reproductive efficiency has attracted considerable attention in recent years.

Economics of Urea-Non-Protein Nitrogen for Dairy Cattle:

One pound of 45% feed grade urea replaces the nitrogen from over 6 lb of 44% soybean meal. The economics of using urea for dairy cattle is obvious when you compare the five cent cost of 1 lb of 45% urea and 5 lb of corn for energy against the 30 cent cost of 6 lb of soybean meal.

That dairymen and cattle feeders alike appreciate this fact is evidenced in the report by Hodges (48) in Feedstuff magazine. He stated that since 1959 world capacity to produce urea had increased five times to an estimated 10 million tons per year by January 1966. United States capacity in the same period increased from 814,800 tons to 1,445,000 tons. In terms of urea use for cattle and sheep, this report stated that urea (calculated as 44% soybean oil meal equivalent) replaced only 12% of the oil meal and grain proteins consumed by sheep and cattle in 1956, but 20% by 1963.

Tokheim (98) reported in Feedstuffs in 1965 that the volume of urea-containing feedlot supplements or feeds had risen about 20% in the previous two year period. He further pointed out the results of a study by the South Dakota Herd Improvement Association where it was estimated that the addition of 10 lb of urea per ton of corn silage saved dairymen milking 60 cows $540 per year in protein costs.

Lassiter (59) in a 1964 talk delivered at the 17th American Farm Research Association conference in Knoxville, Tennessee stated that one Michigan dairyman milking 100 cows claimed an $1800 per year saving in protein costs by adding 10 lb of urea per ton to corn silage.

Dorr (22) reported in 1966 concerning a cost study with Lapeer County Michigan dairymen using urea. This study, involved 12 dairy herds averaging 74 cows each and indicated that a saving of $15.00 per cow in protein costs could be realized by adding 10 lb of urea per ton to corn silage.

There is no question that the use of urea and other non-protein compounds in ruminant diets have tremendous economic appeal. With world food supplies, particularly protein, under increasing population pressure, the use of quality plant and animal protein sources for ruminant needs will become increasingly more difficult to justify. Thus, urea and non-protein nitrogen compounds for protein replacement in ruminants seem made to order in a world of protein shortage for human use.

The Rumen Metabolism of Non-Protein Nitrogen Compounds:

The metabolism of non-protein nitrogen (NPN) compounds plays an important part in reactions in the rumen even when these substances are not added to the diet. To see the utilization of NPN supplements in better perspective, the metabolism of nitrogen in the rumen of animals on unsupplemented rations should be considered first.

The main sources of NPN in the rumen are shown in Fig. 1.

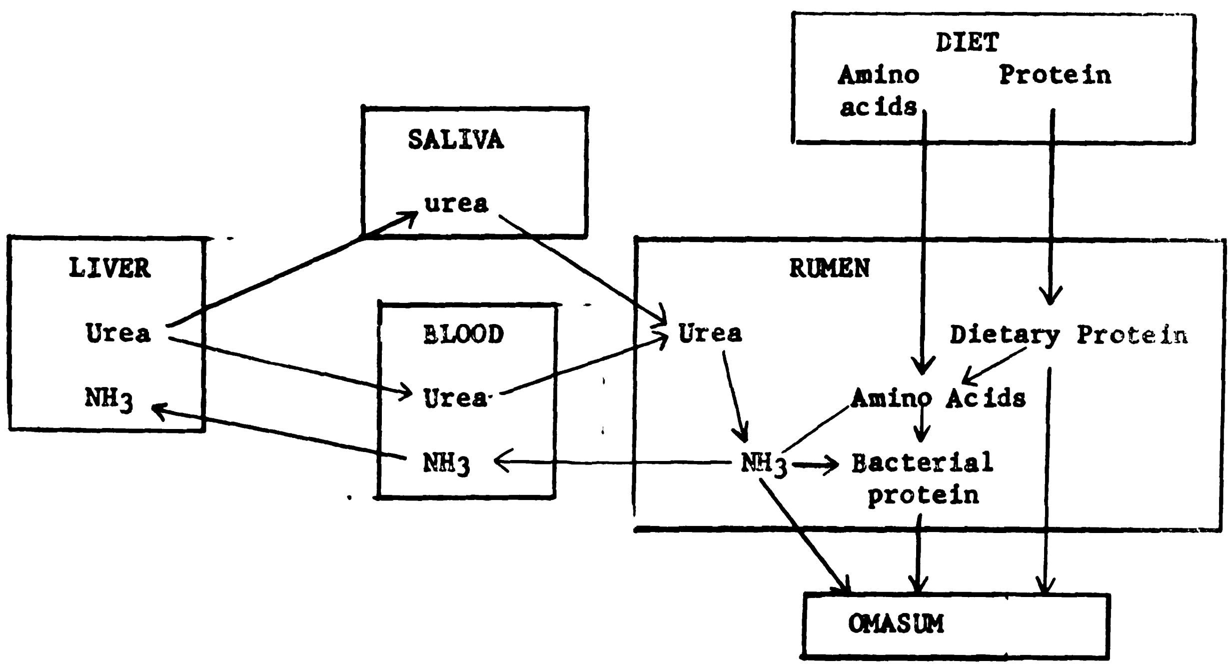

Fig. 1. Sources and fate of NPN in the absence of dietary supplementation.

According to Ferguson and Terry (29) up to 30% of the nitrogen in herbage may be in the form of NPN. These workers indicate that the greater part of this NPN consists of free amino acids, glutamine and asparagine; but small amounts of peptides, nucleic acids, purine and pyrimidine base are also present. A more important source of NPN in the same rumen is the dietary protein which is hydrolysed by the rumen microorganisms to a greater or lesser extent depending on its solubility.

McDonald (65) in studies with sheep found that only 40% of zein was hydrolyzed in the rumen to amino acids. Later studies by McDonald and Hall (66) showed the hydrolysis of readily soluble casein by rumen bacteria was over 90%.

The amino acids released by hydrolysis of dietary protein are rapidly deaminated by the ruminal bacteria and the end products are ammonia, carbon dioxide and organic acids. Microbial breakdown of soluble dietary protein sources may result in very high levels of ammonia in the rumen. Levels of 130 mg of ammonia nitrogen per 100 ml of rumen fluid in sheep have been reported by Johns (57). This is much higher than the ammonia level normally found in the rumen when urea or other forms of supplementary NPN are fed at recommended levels.

Breakdown of Supplementary Urea:

When urea is given as a supplementary source of nitrogen, it is hydrolyzed to ammonia and carbon dioxide similar to endogenous urea in the rumen. This is accomplished by the urease enzyme of rumen bacteria. The hydrolysis of urea in the rumen is unrelated to the ability of the bacteria to utilize the ammonia produced; thus, the concentration of ammonia in the rumen may reach a level which becomes toxic to the animal. Much research in recent years has attempted to overcome this problem by better methods of feeding urea to achieve a controlled release of ammonia.

Thus, in ruminants the rumen microorganisms through urease enzymes, break down urea to ammonia just as they hydrolyze dietary protein to ammonia. From the ammonia and keto acids (which the microorganisms synthesize) rumen microorganisms produce microbial protein. The ruminant then utilizes the microbial protein to meet protein requirements for maintenance, growth, lactation and reproduction.

Certainly the ability of rumen microorganisms to utilize ammonia depends on the simultaneous availability of the other nutrients required for the synthesis of their cellular constituents.

Urea for Growth, Production and Reproduction

From 1940 to the late 1950's the emphasis in research on the use of urea in ruminant nutrition shifted from proof that urea could be used as a replacement for natural protein to determining the extent to which this substitution could be made, and the nutritive factors needed to improve the efficiency of utilization of urea. The object of this research was to determine the value of urea and other non-protein nitrogenous compounds for growth, milk, meat and wool production and the extent to which urea could replace natural protein. The amount of urea which can be used in ruminant rations was found to depend upon the following factors: a) Toxicity level of urea; b) Amount and kind of carbohydrates; c) Amount and kind of true protein.

Beeson (9) as early as 1955 understood the need to supplement urea rations with regard to energy, minerals and vitamins and such recommendations were made to the commercial feeder.

Concern among researchers regarding the safe level of urea use was hightened when Hart et al. (37) in 1939 reported pathological changes in kidneys and livers of dairy heifers fed high urea rations for extended periods of time. Later work by Harris and Mitchell (36) did not reveal liver or kidney damage on even higher levels of urea.

In the late 1940's and early 1950's urea toxicity was of much concern due to a number of accidental deaths of animals being reported. Careless use of urea accounted for most of these deaths, but nevertheless this situation stimulated extensive research on urea toxicity. A review by Gallup et al. (33) of the Oklahoma Agricultural Experiment

Station in 1953 outlined the feeding conditions under which urea toxicity might occur. These were: 1) rapid consumption of a urea-containing feed by non-adapted animals; 2) rapid consumption of urea-containing feeds by starved or fasted animals; 3) rapid consumption of urea-containing feeds by animals on a low protein ration.

Urea for Growth:

It was demonstrated by Brown et al. (17) in 1956 that urea could be used by young dairy calves. Thirty six 2-day-old male and female calves (18 Jerseys and 18 Holsteins) were raised to 86 days on a limited milk-hay starter system to study the value of urea as a source of nitrogen for young dairy calves. The three starters used were: 1) low protein, 6.7%; 2) urea supplement, 15.1% protein; 3) commercial protein supplement, 15.2% protein. Urea supplied 54.2% of the nitrogen in starter 2. All groups of calves grew at comparable rates for the first six weeks during which time whole milk was fed. When milk feeding was discontinued, the urea-supplemented and conventional protein groups grew at a significantly faster rate than the low protein group. In addition these two groups ate more feed and had higher feed efficiency.

Parham et al. (81) in 1955 compared ammoniated molasses, urea and cottonseed meal as sources of nitrogen in rations for dairy heifers. This feeding trial involved 33 dairy heifers and measured daily gains on the three nitrogen feed products. Gains of 1.04, 0.92 and 1.23 pounds per day were produced on the respective sources. Ammoniated molasses and urea supplied 30% of the protein equivalent in the concentrate and 18% of the protein equivalent in the total ration. More feed or energy was required to produce a unit of gain by the animals receiving urea but neither ammoniated molasses nor urea altered the palatability of the feed.

Hart et al. (37) reported work with growing calves where urea, ammonium bicarbonate and casein as nitrogen sources were compared. Calves weighing 250-290 lb were fed rations where 43%, 61% and 70% of the nitrogen came from urea, 69% from ammonium salts and 43 and 66% of the nitrogen from casein. Growth on the calves receiving 43% of their dietary nitrogen from urea was slightly less (1.3 lb gain daily versus 1.5 lb gain daily) than on calves receiving 43% of their nitrogen source from casein. Calves on the higher levels (61, 70, 69%) of nitrogen source from urea and ammonium bicarbonate did not make as rapid gains (0.9 lb gain daily) as did the calves on 43% nitrogen source from urea or casein. The investigators could not conclude whether this difference in gain was due to individual variation or to too high a concentration of the simple nitrogen compounds urea and ammonium bicarbonate.

Repp et al. (92), in experiments with fattening lambs, determined that when urea supplied either 15% or 30% of the protein equivalent growth was comparable to a conventional protein. Urea supplied at 50% of the protein-equivalent, however, did not give comparable gains when compared to soybean protein.

In a growth trial with 108 steers (nine per treatment) Blaylock et al. (12) fed 0, 23, 45, 68, 91, 113, 136, 159 and 181 grams of urea per steer daily for a 112 day feeding period. Animals were fed ground ear corn ad libitum plus 2 lb of hay per day in addition to a protein supplement which contained 32 to 62% crude protein equivalent. Daily gains did not differ significantly between treatments.

Campbell et al. (18), in a study with 40 Guernsey heifers, compared two-times a day feeding with six-times a day feeding where one diet was a 15.3% protein of natural sources and the other diet was a

15.3% protein urea-based diet. The level of urea was 3.3% of the grain ration and 57% of the total nitrogen source. Frequency of feeding the heifers on natural protein diets did not change daily gain significantly; however, feeding the urea diet six times a day versus two times a day resulted in a significant ($P < 0.01$) increase (1.52 lb versus 1.22 lb) in daily gain. The heifers on the urea diet fed six times a day gained 24.6% faster than heifers on the urea diet fed twice a day. These data show that frequent feeding of urea nitrogen compares favorably with protein nitrogen in growth of dairy heifers. Mills et al. (72) worked with Holstein calves and found that with timothy hay as the sole ingredient of the basal ration utilization of urea was low. When molasses provided a suitable substrate for the development of a more active flora urea was fairly well utilized. Adding casein to a 11.6% protein corn molasses urea diet improved growth considerably. These workers concluded that a molasses, urea and timothy hay mixture was not able to provide microbial protein of sufficiently high quality for optimum growth. In the urea-hay group, urea furnished 60-65% of the total nitrogen in the ration.

Milk Production:

Archibald (4) of the University of Massachusetts in 1943 showed that urea nitrogen could supply a considerable portion of the total nitrogen in rations of dairy cattle and not alter production performance. In experiments with cows where protein was supplied from urea, cottonseed meal, soybean meal or corn gluten feed milk production was not significantly different between the urea fed cows and controls, even though 25.4% of the nitrogen in the total ration came from urea. Milk production, however, in this experiment was not at a very high level for any of the ration groups.

Virtanen (101) in 1958 gave cows a dose of ammonium sulphate labelled with ^{15}N and determined the labelling of the amino acids separated by fractionation after they hydrolysis of the milk protein. It was shown that in the milk obtained 15 hours after feeding the labelled compound all the protein amino acids studied were labelled, but that the degree of labelling was different.

Reid (91) in 1953 reviewed the use of urea as a protein supplement and stated that urea could effectively substitute for plant protein when fed at levels of not more than 27% of the total ration nitrogen.

In 1943 when a small amount of crystal urea was made available under allocation to feed manufacturers, Hastings (46) of Lindsey Robinson and Co. of Roanoke, Virginia conducted experiments with Holstein milk cows to determine if urea could be used as a protein replacement source in commercial dairy feeds. Experiments covering 210 days where urea non-protein nitrogen made up 36% of the total in the diet Hastings concluded at least, over the short period of the test, that urea could be used in dairy rations to replace part of the natural protein without unfavorable results. Hastings further concluded that milk production on the urea group was maintained and that milk fat and milk protein in the urea produced milk was comparable to the milk in control cows. Milk production, however, in these experiments was just average.

With regard to the proper percentage of total diet nitrogen that could be supplied from urea there have been conflicting reports in the literature. Stangel (95) in 1963 reviewed the use of urea and other non-protein nitrogen compounds. He cited numerous experiments and suggested that urea could be used successfully as a nitrogen extender when the urea nitrogen supplied no more than one-third of the protein equivalent in ruminant rations. However, in the light of more recent data this may not be true for high producing cows, particularly, when the urea is fed through the concentrate.

Huber et al. (53) added varying amounts of urea at the time of feeding to rations for high producing cows. Ninety-one lactating cows were involved in three experiments. The forage supplied was corn silage fed ad libitum. From 0 to 48% of the total dietary nitrogen was supplied from urea. When 11% of the nitrogen came from urea no adverse effects on milk production was observed. However, when urea furnished 21 to 23% of dietary nitrogen milk yeilds were depressed significantly. When urea supplied 38 and 48% of the ration nitrogen a more radical depression in milk production was noted. From these studies the authors concluded that the maximum urea that can be added to concentrate for high producing cows without depressing milk yield is about 180 grams/day. They further suggested that the old recommendations of furnishing one-third of the dietary nitrogen as urea was not applicable to cows at high production levels where protein needs are greatly increased.

Flatt et al. (31) reported experimental results where a cow fed a purified diet containing urea as the source of nitrogen produced 2630 kg of milk while her monozygotic twin fed a natural diet produced about double this amount during a 305 day lactation. During a second lactation, milk production of the cow receiving the purified urea diet was about 65% of that of her twin. The metaboliza b le energy of both diets was used similarly and this was true regardless if protein or non-protein nitrogen was the source of dietary nitrogen in the purified diet.

Van Horn et al. (100) in recent experiments (1967) challenged the older concepts that urea could supply 35% of the total nitrogen in the ration, could constitute up to 3% of the concentrate mixture and could constitute as much as 1% of the total ration dry matter. He emphasized that most of the literature reviewed whose data arrived at those conclusions dealt with cows producing less than 20 kg milk daily. Van Horn conducted several experiments to deal with this problem. In an experiment with 20 lactating Holstein cows concentrate acceptability was studied. Additions of 2.2% and 2.7% urea and 15.9% and 19.0% corn cobs to the concentrates significantly ($P < 0.01$) depressed intake, but there was no interaction between urea and cobs. Milk production appeared to be directly related to concentrate intake. ($P < 0.01$).

Van Horn et al. (100) reported an additional experiment where 24 lactating Holstein cows were divided into three treatment groups. Group 1 served as the control and groups 2 and 3 received silage to which was added 5.0 kg of urea (45%) per metric ton at the time of ensiling. Group 2 received urea only in the silage while group 3 received urea in silage plus 1% urea added to the concentrate mixture. This experiment lasted 80 days following a 10-day standardization period. The results of this experiment showed no difference between these three groups in hay, silage or concentrate consumption and no difference in body weight gain or milk or fat production. These workers concluded that inclusion of 1% urea in the concentrate was acceptable to dairy cows without depressing feed intake. They further pointed out that one of the main advantages of adding urea to corn silage was that it allowed use of higher levels of urea in the total ration than could be included in the concentrate alone.

These reports suggest that urea can supply a substantial portion of the dietary nitrogen, the amount depending on the ration make up, method of feeding and whether the ration is for growth or production. Rupel et al. (93) gave guidelines for feeding urea which are generally accepted today; mainly, that urea make up not more than 3% of the concentrate mix, be less than 1% urea in ration dry matter, and not comprise more than one-third of the protein equivalent in the total ration. The guidelines set by Rupel et al. (93) however, were based on experiments were milk production was low by today's standards. More recent research by Huber et al. (53) with high producing cows indicate that when urea added to the concentrate furnished 22 to 25% of the total dietary nitrogen with corn silage as the principal forage, milk yields were depressed. Huber pointed out that satisfactory growth of dairy herd replacements fed urea-treated corn silage as the only feed has been noted (Schmutz, 1966, J. T. Huber, unpublished data). Huber further noted that decreased palatability of concentrates has been reported when the urea content exceeded 2%.

For more comprehensive and detailed reviews of the utilization of urea and its effect on performance the reader is directed to the following more recent reviews: 1) Reid, J. T. (91); 2) McLaren, George A. (67); 3) Chalupa, William (19); and 4) Huber et al. (52).

Reproduction:

Urea used as a protein replacement for growth and production in the rations of cattle and sheep has been studied in numerous experiments for many years. Since the nitrogen in urea is metabolized in the same manner as the nitrogen of dietary protein it would not seem that use of this compound should have any adverse effect upon reproductive efficiency in dairy cattle or other ruminants. However, most of the experiments with urea have been under controlled research conditions without the interacting effect of management, environment and high production. Most of the studies have been designed as nutrition experiments and reproduction information was not noted or was limited in detail.

Campbell et al. (18) reported that if urea is metabolized too rapidly, and the rumen microorganisms cannot convert the ammonia produced into microbial protein at a fast enough rate then ammonia levels in the blood will increase and urea is used inefficiently. Experiments by Campbell et al. (18) suggest that the ammonia in the blood is converted to urea by the liver to be eliminated by the kidneys or can re-enter the rumen via the saliva or by direct passage through the mucosa of the gastric reservoirs. How borderline levels of blood ammonia interacts with vitamin utilization and hormone levels has not been studied conclusively and remains subject to further investigation and understanding.

In reviewing past experiments where reproductive performance was noted, the increased services per conception reported might well be a factor in lengthened calving interval and reduced breeding efficiency. Should vitamin A utilization be altered as some researchers are inclined to believe, this might well have an effect upon reproductive efficiency.

Rupel et al. (93) conducted some early nutrition studies in the use of urea in dairy rations. These workers not only compared the nutritive value of urea versus linseed meal but in addition noted reproductive performance of cattle fed urea diets. This experiment was conducted with 24 grade Holsteins, fresh from 5-13 weeks and carried through an entire lactation. Rupel et al. (93) noted in their report that a study of the records showed no disturbing effect of urea on the estrus cycle or the frequency of service to obtain pregnancy. The actual services-per-conception information was not detailed in this study. The concensus of these workers was that when urea supplied half of the protein-equivalent in an 18% protein ration breeding efficiency was not altered.

Archibald (4) in 1943 worked with urea feeding in dairy cattle in a nutrition study involving two groups of Holsteins over two complete lactations. Reproductive performance was not the purpose of this study but was indicated to be comparable for the urea and non-urea fed groups.

Miller et al. (71) of Oklahoma in a Feeder's Day Report in 1958 discussed urea protein supplements for wintering fall calving cows. This group working with brood cows did not note any deterimental effects of urea feeding on reproduction, calving or breeding efficiency.

Bates et al. (6) conducted experiments with two groups of Holstein and Jersey heifers using a liquid urea molasses mineral mix with added ethyl alcohol compared to the same ration without the alcohol. Two groups of ten heifers each were fed these two liquid mixes ad libitum plus ground corn cobs fed separately ad libitum for 140 days. One Jersey heifer was continued on each of the rations for an additional 483 days. Reproductive performance on these two animals was noted. The heifer on the urea-liquid mix without added alcohol produced a 42 lb calf which died a few days after birth. The heifer from the liquid-urea mix containing 6% alcohol produced a 37 lb calf which lived. These investigators concluded that the only apparent nutritional inadequacy during this extended time period was energy.

Bond et al. (13) of the United States Department of Agriculture, Beltsville, Maryland in a replicated experiment with six pairs of identical twin Angus heifers, compared growth and reproduction on a poor quality, 50% forage diet where two-thirds of the total nitrogen was supplied either as soybean meal, one-half soybean meal, and one-half urea, or urea. Corn starch and urea were used to replace the soybean meal in the diet. The diets were fed on an isocaloric and isonitrogenous basis. In this experiment with two replicates (started one year apart) diets had no significant effect on services per conception, length of gestation period (days), birth weight of calves or number of days from calving to first estrus. However, the length of estrous cycle was slightly longer ($P < .10$) in the heifers receiving urea (22.2 days) compared to 21.3 days and 21.4 days for the other two groups.

Pope et al. (86) fed ewes in gestation a natural protein diet and obtained an average of 18 lb of gain per ewe during gestation. When the grain ration of natural protein was raised to 14% with urea or cottonseed meal the gestation gain was increased to 25.6 and 26.9 lb respectively. The average birth weight of the lambs produced on the three rations was 9.8, 10.6 and 10.4. The use of urea over natural protein for the grain rations at the 14% protein level had no apparent effect upon weight gain of the ewes in gestation or the birth weight of the lambs.

In 1952 Pope et al. (87) reported further work on the value of urea for ewes during gestation and lactation. Three gestation grain rations were fed: 10.3% crude protein from natural sources, 15.6% protein-equivalent urea diet and a 15.3% protein diet from cottonseed meal. Lambs born of ewes fed the control ration (10.3% crude protein) gained 20.3 lb during the first 42 days following birth, whereas gains of 24.3 and 25.1 lb were obtained during the same period by lambs produced by ewes fed urea-containing pellets and high cottonseed meal pellets. The ewes were fed 2 lb of prairie grass hay daily during pregnancy and lactation, and 1 and 2 lb respectively of the pelleted concentrates. In this experiment no significant differences were found among the birth weight of lambs, the fleece weights or changes in the weight of the ewes during lactation.

Virtanen (102), in his classic work of feeding several cows over an extended period of time protein-free diets where non-protein nitrogen urea and ammonium salts served as the sole nitrogen source discussed reproduction.

Virtanen noted that the estrus cycle of the test cows was regular and easily observable and all cows became pregnant. It was observed, however, that many of the test cows required several services to become pregnant. Admittedly this work was on small numbers of cows, but if several services are required on high urea rations to achieve pregnancy then calving interval would be lengthened and breeding efficiency reduced.

Oltjen and Bond (76) in 1967 conducted experiments with reproduction where Angus and Shorthorn twin heifers were raised on a protein free diet. These workers used a set of Angus monozygotic twin heifers and a set of Shorthorn dizygotic twin heifers weighing 140 kg. The heifers were seven months of age when started on test. One heifer from each set received the urea purified diet and the other a natural protein diet. The heifers were fed on an isocaloric and isonitrogenous basis. This experiment was terminated when the heifers were 20 months old. Oltjen indicated that though the data was limited there appeared to be a slightly shorter estrous cycle, a longer gestation period and more services were required per conception with heifers fed the urea diet. There were two abortions by the Angus cow and one by the Shorthorn cow fed the urea diet.

In a later study Oltjen et al. (76) conducted a more complete growth and reproduction experiment using 28 bull and heifer calves. These animals were offered purified diets containing either urea or isolated soy protein or a natural diet ad libitum starting at 14 days of age. They were weaned onto the diets at 84 days of age to determine the long term effects of urea on growth and reproduction. Bulls fed urea reached puberty (measured by semen characteristics) two months later than the bulls fed the soy and natural diets. The urea fed heifers reached puberty (first estrus) approximately six months later than the soy and natural fed heifers. These workers concluded that when urea was used as the sole source of dietary nitrogen for ruminants, growth and nitrogen retention was about 70% of that when either purified protein diets or natural diets are fed. In this experiment the slower onset of puberty could probably be accounted for by the slower growth rate on the urea purified diets.

Holter et al. (49) reported in 1968 of experiments with urea in the concentrate of high producing cows where conception information was recorded. A high producing herd of 56 purebred Holstein cows were divided at random into two groups. One group was fed a premium commercial concentrate containing no urea during the 18 month experimental period. The other group was fed a similar concentrate mixture in which urea nitrogen replaced plant protein nitrogen to the extent of 3.9 percentage units of equivalent crude protein. According to these workers mean conception rates in the urea and control cows were 1.6 and 1.9 services per conception respectively. The proportion of cows requiring one, two or more services for conception were not statistically different ($P > .10$). These researchers concluded however, that the 18-month experimental period was not sufficiently long to permit a firm conclusion about the effect of feeding urea on conception.

Holter point out, however, in this study that the maximum amount of urea that a cow could get per day was 170 grams and that the average amount received per cow per day over the entire feeding trial was considerably less than this amount.

Boyd (14), in an article for the popular press, discussed the issue of whether urea feeding altered fertility in dairy or beef cattle. He reviewed eight experiments and concluded that there was no experimental evidence indicating that urea caused poor reproductive performance in cattle.

In the experiments reviewed thus far many of the reproductive disturbances noted were concluded to be due to the reduced growth of the animals brought about by lessened nitrogen retention on the all-urea-equivalent protein diets. Many of the growth and production experiments reported were with animals where the total intake of urea in grams per day was not of a high order. Under practical farm situations dairymen might feed urea far in excess of recommended amounts or of the amounts reported in the experimental studies. What effect these higher levels of urea might have on services per conception, vitamin A utilization or other interacting nutrition factors could be of prime importance in understanding the role of non-protein nitrogen compounds like urea in reproduction efficiency.

Several of the experiments reviewed reported that it required more services per conception to obtain pregnancy where animals were getting substantial amounts of urea. This experimental observation describes the practical field situation where dairymen report difficulty in getting cows settled when feeding substantial intakes of urea.

Urea and Vitamin A Status:

Another most important nutritive factor that has been studied in connection with urea use and reproductive performance is the affect of urea upon vitamin A status and utilization. The importance of this vitamin and reproduction has been discussed earlier. It is generally known that vitamin A nutrition is influenced by a wide variety of factors including the nature of the diet, and it has been shown that a number of dietary materials affect the metabolism of carotene and vitamin A. Moore (73) in his 1957 review of the literature on vitamin A did not refer to any studies of the relationship between vitamin A nutrition and utilization of dietary urea. There were numerous instances in the review, however, which implicated protein nitrogen and nitrogen metabolism in vitamin A nutrition. In Stangel's (95) 1963 review of research with urea and non-protein nitrogen in nutrition, of 1535 articles cited less than half a dozen concerned the relationship of dietary urea and vitamin A nutrition. Though limited in number these urea-vitamin A experiments should be reviewed for possible clues to reported reproductive difficulties in cattle, whose diets contain considerable amounts of urea.

Gallup et al. (32) studied the comparative effect of several sources of dietary protein on vitamin A metabolism in sheep. In these experiments comparable groups of sheep which had been partially depleted of their stores of vitamin A were fed low carotene rations containing soybean oil meal, cottonseed meal and urea as the principal sources of nitrogen. In follow up experiments these same sheep were given supplements of high potency cod liver oil, prairie hay and a carotene concentrate as sources of vitamin A or carotene. Vitamin A content of the blood was determined periodically. Vitamin A storage in the liver was determined at the beginning and end of the supplement-feeding period. Five experiments were conducted. The authors concluded from these experiments that urea was without effect on carotene and vitamin A metabolism under the conditions of their experiment.

Smith et al. (94) studied the influence of urea upon vitamin A nutrition of ruminants. In their 1964 experiments Smith and co-workers fed sheep semi-purified diets and cattle were fed fattening diets some of which containted urea as a major source of nitrogen. Vitamin A status was evaluated in terms of plasma and liver vitamin A. Several experiments were conducted by Smith and co-workers and reported in 1964.

In the initial experiment, 15 feeder lambs of 80 to 100 lb in body weight were fed for 97 days a semi-purified diet containing urea as practically the sole source of nitrogen. The diet was consumed at the rate of 2.2 lb per lamb daily and supplied vitamin A palmitate at 15,000 international units (I.U.) per lamb daily. After 97 days liver samples were obtained by biopsy. Vitamin A concentrations in the liver varied from 14 to 125 micrograms per gram of fresh liver. The mean of the values, 68 ug/g, was considerably below the value that is expected from lambs having received 15,000 IU daily for this lengthy period. The estimated requirement for sheep of this size is approximately 1000 I.U. daily (N.R.C.) and the average of liver vitamin A concentraions in a wide range of normal sheep is approximately 150 ug/g. In this experiment the wide range in liver vitamin A and large variation (standard deviation - 4 ug/g) for sheep uniformly treated for so long was both unusual and unexpected. Body weight gains of 0.04 lb per head daily were poor and were improved markedly when soybean protein was provided in a diet otherwise very similar. These workers suggested that a protein deficiency may have been a primary factor affecting both vitamin A status and weight gain.

In a second trial with lambs from Experiment 1, Smith et al. (94) attempted to determine whether excess urea in the diet would influence the vitamin A nutritional status under circumstances where protein nutrition was not dependent upon synthesis of protein from urea nitrogen. Two groups of 12 lambs were used, and each group received a diet containing purified soybean meal as 12% of the diet; however, one of the diets contained additional urea as 5% of the diet added in replacement of corn starch. Diets were fed at 2.2 lb per lamb daily providing 15,000 I.U. vitamin A (palmitate) daily for 30 days. Upon slaughtering, samples of blood and liver were analyzed for vitamin A and carotene. Body weight gains for the two groups of lambs were comparable as were the mean values for plasma vitamin A. Livers of lambs fed the non-urea diet markedly increased in their concentration of vitamin A; whereas, livers of lambs fed urea showed no appreciable change in vitamin A concentration. The change in vitamin A concentration during the trial (30 vs 3 ug/g) differed significantly ($P > 0.01$) suggesting that urea impaired the liver storage of vitamin A. However, livers of lambs fed urea were larger (560 g vs 454 g, $P > 0.05$) with the result that in terms of total content of vitamin A in the livers, the two groups did not differ.

Additional experiments were conducted by Smith and his group with steers and rats, and their conclusion was that even at excessive intake levels urea had no noticeable adverse effect upon either the mobilization or storage of vitamin A. It must be remembered that these experiments were of limited number and that none of them concerned high production milking cows with urea intake coming from several sources under practical farm feeding conditions. That there is a relationship between dietary nitrogen, from protein or urea, and vitamin A status and utilization seems evident. Whether these factors play a role in reproduction performance remains unclear.

Patton et al. (83) reported an experiment where urea was fed to 27 Holstein heifers for 2 months before breeding to provide 45% of the dietary nitrogen. A control group of equal number received all nitrogen as plant protein. Reproductive efficiency, rate of gain, blood progesterone, and blood urea were compared. Services per conception were not different and blood urea was the same. This research is a continuing study but at this point no reproductive effects from urea were observed.

A Michigan State University laboratory study Britt et al. (16) (unpublished) conducted work with different urea levels in the diets of female rats. Forty female Albino rats of breeding size were divided into four treatment groups. The control group received a standard 20% protein rat diet while the remaining three groups received the same diet to which 1.5, 3.0 and 6.0% urea was added. After a 2-week adaptation period male rats were placed in the pens. Half of the female rats were slaughtered and posted for observation at day 10 of pregnancy and the remaining half at day 18. Three observations were noted: 1) Number of days required to achieve pregnancy as determined by vaginal smears and sperm observations; 2) number and condition of fetuses; 3) number of corpora lutea. Looking only at the 20 rats slaughtered at day 10 (the day 18 rats were biased due to mating procedures) of pregnancy the results indicate that urea significantly increased the number of days required for conception to occur from 2.2 days for controls to 8.4 days for the females fed 6% urea. This was significant at $P < 0.05$ or pn 0.04. The number of normal fetuses was reduced from 10.2 in the control group to 5.2 in the 6% urea group.

This experiment was of limited scope but the procedures were accurate and carefully supervised. Urea should not have any effect upon the performance of monogastric animals like rats since it is known that practically all of the urea consumed by rats is excreted in the feces and urine. The implications of this reduced breeding efficiency in rats on high urea diets cannot be explained by this limited evidence.

Rats and ruminants are vastly different kinds of animal but limited experimental evidence and increasing field reports claiming a urea and breeding efficiency link seems to warrant further investigation into this problem area. The very fact that the effect of feeding urea on reproductive efficiency has not been thoroughly tested under practical farm conditions is probably the single most important factor confirming the need for this study.

EXPERIMENTAL PROCEDURE

To test the hypothesis that feeding urea to dairy cattle affects the calving interval, Dairy Herd Improvement Association herds in Michigan were studied.

Calving interval measured as the number of days from parturition to parturition has been used to estimate breeding efficiency. Legates (61) reported a mean calving interval of 406 days on 2,419 records. Hutchinson et al. (56) found a mean calving interval of 392 days. A calving interval of 453 days on 629 cows was reported by Davis (21). The disadvantage of calving interval as a measure of breeding efficiency would be the management's decision to intentionally extend the time waited from parturition to first service. Armstrong (3) stated that calving interval was a good measure of breeding efficiency provided that management factors affecting length of calving interval could be taken into account. If the breeding philosophy of the dairyman in terms of the number of days waited from parturition to first service was known, then an adjusted calving interval could be calculated on a herd basis and used as an effective measure of breeding efficiency. In this investigation an adjusted calving interval was used to measure the breeding efficiency of the Dairy Herd Improvement Association herds included in the final sample. Herds not having used urea served as the control herds in this study.

Statisticians indicated that if four levels of urea intake were compared, it would require 65 average sized herds in each group to significantly show a five-day difference in calving interval. Thus, 260 Michigan dairy herds of about 10,000 dairy cows were needed in this study.

To study the accumulative effects of urea feeding the five year period of October 1964 through September 1969 was chosen as the time period for the procurement of dairy feeding practices, urea use, and breeding information.

A survey questionnaire sent to Michigan Dairy Herd Improvement Association members provided the necessary information needed for this study and on a sufficient number of herds and dairy cows to make the results meaningful.

<u>The Survey Instrument</u>

To properly evaluate the influence of feeding urea upon calving interval the survey questionnaire was designed to provide the following nutrition, reproduction and factual information concerning the herds to be analyzed and for the time period involved:

1. Average level of grain being fed per cow per day in each of the years of the study.
2. Average level of silage being fed per cow per day and number of months fed for each of the years of the study.
3. Mineral and vitamin A feeding practices.
4. Sources of urea in the ration and the amount being added to corn silage, grain or complete dairy ration.
5. The brand of commercial supplement being used, whether it contained urea and how much commercial protein was being added per ton of milking ration.
6. The breeding philosophy of the individual dairy man in the study in terms of the number of days after calving waited until re-breeding procedures were initiated.

7. Information concerning the attitude of the dairmen about urea use and its overall affect upon his herd performance.
8. Pertinent information about the dairymen's artificial insemination practices.
9. Information about the dairyman that would identify his location and number of years in the Dairy Herd Improvement Association record system.

A survey questionnaire was developed and field tested. Necessary changes were made with three criteria in mind: 1) length should be confined to three pages; 2) questions be such that they could be answered easily and quickly; 3) nutrition and reproduction facts cover a five year period, and be such that an adjusted calving interval could be calculated and the average level of urea intake from all sources per cow per day on a herd basis could be easily determined.

Three levels of grain feeding were reported by farmers. The three feeding levels chosen were: 1) low grain-less than 10 lb per cow per day; 2) medium grain - 10-14 lb per cow per day; 3) high grain-more than 15 lb per cow per day.

Silage feeding practices were reported as: 1) no silage; 2) 20-40 lb per cow per day; 3) 40-60 lb per cow per day; 4) silage fed free choice. In addition the dairyman reported the number of months in the year corn silage was fed.

Vitamin A and mineral feeding information was reported only on the basis that supplemental minerals or vitamin A were or were not being fed.

Urea addition to corn silage, high moisture corn or dry grain was reported in pounds of urea added per ton of the feed ingredient.

Commercial supplement information was reported on the basis of brand name of supplement, whether it contained urea, percentage of protein and amount added to make a ton of milking ration. This information enabled the investigator to determine the grams of urea per pound of feedstuff and from this calculate the average urea intake per cow per day on a herd basis.

Herd breeding philosophy information was reported by the dairymen simply as the number of days waited after parturition until re-breeding procedures were initiated. A copy of the survey instrument is included following this section.

Distribution and Collection of the Survey Instrument:

Local County Agricultural Extension Agents distributed the survey instrument to county Dairy Herd Improvement Association members and handled the collection and return of the completed questionnaires. Where needed, re-survey procedures were handled in the same manner.

Final Sample Selection:

The parameters for inclusion of a completed questionnaire in the final sample to be studied were:

1. Survey information must be complete and accurate as viewed by the investigator.
2. Herds included in the non-urea use category be herds where no urea had been used during the five year period under study.
3. Final sample include in the urea use group a high percentage of herds that had used urea in some manner for at least three of the five years of the study period.
4. For calving interval data herds included in the final sample must have been in Dairy Herd Improvement Association membership in at least four of the five years of the study.

5. Breeding philosophy information relative to calving interval adjustment must be available on all questionnaires included in the final sample.

Computer Card Set-Up:

A computer card was prepared for each herd for each of the five years included in the study. These were identified by DHIA herd number and by year. The following data from each of the final sample questionnaires by years was placed on the computer cards:

1. Herd number and year.
2. Daily grain intake in pounds per cow per day.
3. Daily corn silage intake in pounds per cow per day.
4. Number of months in year corn silage was fed.
5. Whether supplemental minerals were fed.
6. Whether supplemental vitamin A was fed.
7. Daily intake in grams of urea per cow per day from corn silage.
8. Daily intake in grams of urea per cow per day from high moisture corn.
9. Daily intake in grams of urea per cow per day from dry grain additions.
10. Daily intake in grams of urea per cow per day from commercial supplement.
11. Total daily intake in grams of urea per cow per day from all sources.
12. Information as to whether the farmer knew that his supplement contained urea. This was determined by whether he answered correctly, incorrectly or did not know whether his commercial supplement contained urea.
13. Information as to his opinion whether urea caused reproductive difficulties and what his attitude (favorably, unfavorably or no response) was regarding urea use.
14. Information regarding whether he had quit using urea during the survey period and for what reason.
15. The number of days the responding dairyman, as a herd policy, waited before initiating re-breeding procedures.

With the information from the final sample herds on computer cards the urea use and calving interval differences could be studied and evaluated.

Calving Interval and Adjustment:

Dairy Herd Improvement Association computerized records were used to determine the average length of calving interval for each herd in the final sample. This calving interval was adjusted by the number of days waited after parturition for re-breeding procedures to begin as reported by dairymen whose herds were included in the analyses sample.

NUTRITION REPRODUCTION SURVEY

Name of Producer ______________________ Address ________________

____

County ________________________ DHIA or Owner Sampler Number _____

Number of years on DHIA _____________ Owner Sampler _____________

Which of the preceding years were you on DHIA for full year (✓check):

1968-1969 _____ 1967-68 _____ 1966-67 _____ 1965-66 _____ 1964-65 _____

Nutrition Facts:

For each of the past five years, rate your winter* grain feeding practices (Mark an x in the appropriate column).

Year	HIGH GRAIN**		MEDIUM GRAIN**		LOW GRAIN**	
	1# grain to 2# milk	15# or more grain per cow/day	1# grain to 3-3½# milk	10-14# grain/ cow/day	1# grain to 4# milk	Less than 10# grain/ cow/day
1968-69						
1967-68						
1966-67						
1965-66						
1964-65						

*We are interested in the average amount of grain fed per cow per day on a herd basis during the fall, winter feeding season.

**Under grain levels the two choices represent about the same volume of grain fed. Mark whichever column is most consistent with your feeding program.

Do you feed corn silage? Yes - No (circle). Rate your corn silage feeding practices in the past five years. (Mark an x in the appropriate column or circle correct number.)

Year	No corn silage fed	Corn silage fed free choice	40-60# fed per cow/day	20-40# fed per cow/day	No. months in year corn silage fed
1968-69					2 - 4 - 6 - 8 - 10 - 12
1967-68					2 - 4 - 6 - 8 - 10 - 12
1966-67					2 - 4 - 6 - 8 - 10 - 12
1965-66					2 - 4 - 6 - 8 - 10 - 12
1964-65					2 - 4 - 6 - 8 - 10 - 12

Do you feed minerals? Yes - No (circle). In grain mix? Yes - No (circle). Free choice? Yes - No (circle).

Do you feed a supplemental vitamin A in your grain mix to your cows? Yes - No (circle).

SILAGE AND GRAIN FEEDING PRACTICES

This section is to determine where and how much urea was used in your dairy feeding program in the past five years.

Part 1. Silage - Grain: (Mark an x in proper space or fill in amount - where no urea was used please indicate by placing an x in the appropriate no columns).

Feed-stuff	1968-69 Urea added		Amount added/ton*	1967-68 Urea added		Amount added/ton*	1966-67 Urea added		Amount added/ton*	1965-66 Urea added		Amount added/ton*	1964-65 Urea added		Amount added/ton*
	Yes	No		Yes	No		Yes	No		Yes	No		Yes	No	
Corn silage															
High moisture corn															
Dairy** grain ration															

*Where urea was used be sure to give amount added per ton.
**Refers to dairy milking ration where straight urea is added.

Part 2. Protein Concentrate: (Mark an x in proper space or fill in blank). Please fill in this section completely even though protein supplement did not contain urea.

	1968-69		1967-68		1966-67		1965-66		1964-65	
	Yes	No	Yes	No	Yes	No	Yes	No	Yes	No
Contained urea										
Brand* of protein conc.										
Protein % of concentrate**	___%		___%		___%		___%		___%	
Amount added per ton of ration**	___#		___#		___#		___#		___#	

*If commercial protein concentrate, list brand name - if protein ingredient, name ingredient (Soy, linseed, etc.).
**Be sure to give percent protein and amount of protein concentrate used to make ton of milking ration.

REPRODUCTION FACTS:

1. Do you use artificial insemination? Yes - No (circle).

2. What percent of your herd in previous 5 years was bred by A.I.?
1968 _____ 1967 _____ 1966 _____ 1965 _____ 1964_____

3. As an overall herd policy how soon after calving do you start rebreeding cows? ____________________ (Indicate number of days).

4. Do you ever rebreed any cows before 50 days after calving? Yes - No (circle). If so, what percent of your herd is bred by 50 days after calving? ____________

5. Does the length of time that a cow has been fresh make any difference as to when you start rebreeding after calving? Yes - No (circle).

6. Do you deliberately wait 90 days or more after calving before rebreeding any cows? Yes - No (circle). If so, what percent of your herd would be held open for 90 days or more before you start rebreeding? (______%).

7. Any other comment concerning your rebreeding policy? ____________

____

____

____

____

8. How many days dry period do you allow for the cows in the herd?
____________ days.

____

9. Do you have other comments or observations concerning urea use in your dairy feeding program?

____

____

____

____

RESULTS AND DISCUSSION

The response to the information questionnaire sent to dairymen by county extension personnel was excellent. Of 1400 Dairy Herd Improvement Association member herds, a total of over 900 completed questionnaires were obtained. Over 700 of these herds were used in the final sample analysis, and this represented some 85,281 individual cow calving intervals.

A total of 3157 herd observations were included in the data from Fairy Herd Improvement Association records and survey forms from the years 1965 through 1969. A summary of the means, range and standard deviations of the variables studied is presented in Table 1.

Number of cows per herd ranged from 1 to 1247 and averaged 48.6 cows. Milk production (305 day) ranged from 5,506 to 20,074 pounds and averaged 12,964 pounds per cow for the five-year period. The average adjusted calving interval (ACI) was 315.5 days and ranged from 244 to 496 days. Days from calving to first breeding averaged 66.2 days with a maximum of 99 days as herd re-breeding policy indicated by the herd owners. Combining the above two items results in an average calving interval of 381.7 days or 12.5 months. The actual calving interval for each group is shown in Table 2.

Urea was fed in 1709 of the 3157 herd observations which comprised 54.1% of the cases. Since the data in Table 1 represen the pooled data of herds fed no urea and those fed urea, the means and standard deviations as grams of urea fed per day are misleading. When only the number of herds fed urea is used as the denominator the average amount of urea fed per cow daily from corn silage was 39.9 grams, from high moisture corn 0.6 gram, from dry grain 7.5 grams, from commercial protein supplements 32.6 grams, and the average total urea from all sources was 80.6 grams per cow daily. The maximum amount of urea fed to any herd was 370 grams per head daily.

The average adjusted calving intervals for herds fed no urea, or urea (all levels) for years 1965-1969 and percent of herds fed urea are shown in Table 3. From these data it appears that the adjusted calving interval for herds not fed urea as well as those fed urea was slightly higher for years 1967 and 1969. The magnitude of increase in (days) adjusted calving interval (ACI) was approximately the same for herds fed no urea as for those fed urea, in both years. The low correlation between years and ACI ($r = .06$), although statistically significant ($P < .001$), suggests that the magnitude of relationship is not sufficient to be of any practical importance for assuming effects of years on calving interval.

<u>Relationship of Urea to Calving Interval</u>

The effect of various levels of urea fed on calving interval, adjusted calving interval, and percent of cows sold for sterility, along with other data, is shown in Table 2.

Calving interval and adjusted calving interval were not significantly different for herds fed various levels of urea compared to herds fed no urea. The mean adjusted calving interval for all herds fed urea was 315.7 days versus 314.4 days for herds fed no urea. Herds fed 1-60 grams and 181-370 grams urea had a lower adjusted calving interval than herds fed no urea, while other urea levels had slightly longer but not significantly different calving intervals.

Milk Production:

Average 305-day milk production information for the herds fed no urea and those fed various levels of urea is reported in Table 2. These differences were not significant and probably are unrelated to the feeding of urea. Those herds fed the highest levels of urea tended to have the highest milk production.

Length of calving interval was not related to either the source or level of urea fed in this study. The simple correlation (Table 4) between total urea intake (grams/day) from all sources and adjusted calving interval was 4 = .03 and nonsignificant. Similarly, the partial correlation coefficients for the various sources of urea were: urea from corn silage (4 = -.01), urea from high moisture corn grain (4 = 0.007), urea from dry grain (r = .02), urea from commercial concentrate supplements (4 - .02). None of these correlations were significant.

Number of years urea was fed and level of urea were not related to adjusted calving interval as shown by the low correlation (r = .01) for year X urea interaction and calving interval. Further evidence that urea levels were not related to calving interval is shown by the low correlations between the urea square (r = -.008), cubic (r = .01), and quadratic (r = .01), equatious with calving interval.

If curvilinear relationships between levels of urea and calving interval had existed one or more of these correlations would have been significant. The simple correlations between the nineteen variables studied are shown in Table 4.

The multiple correlation coefficients (R^2 = .154) indicate that only about 15 percent of the total variation in adjusted calving intervals was accounted for by the nineteen variables included in the study.

While none of the sources of urea were significantly correlated with adjusted calving interval it is noteworthy that several of the other variables were highly significant even though their correlation coefficients were small. Year of the observations, months corn silage was fed, percent of cows in herd bred by artificial insemination, and number of cows in the herd were all positively related to the length of adjusted calving interval while days of intentional delay from calving to first breeding and pounds of milk per cow were negatively related to adjusted calving interval.

The negative relationship between days from calving to first breeding and adjusted calving interval (r = -.37) was largely due to the adjustment procedure.

If one assumes that there is no effect on conception rate by delaying first breeding then the real calving interval would be increased by delaying first breeding while the adjusted calving interval is decreased by delayed breeding, i.e. subtracting more days from real calving interval results in proportionately less days for adjusted calving interval. The computed automatic part of the correlation between days of adjustment and adjusted calving interval (ACI) is r = -.52, while the correlation between real calving interval and days of adjustment is positive (r = .14) thus the negative correlation (r = -.37) is largely a function of the adjustment procedure.

The real calving interval tended to be increased by delay in breeding when the automatic negative effect due to adjustment procedure is removed. In fact this variable comprised 87 percent of the total variation (R^2 = .151) in adjusted calving interval accounted for by all the variables that were statistically significant.

Least Squares Deletion

Submission of the partial regression and correlation data to a least squares deletion procedure resulted in deletion of variables as related to adjusted calving interval in the following order: (1) Grain, (2) vitamin A, (3) urea from high moisture corn, (4) $Urea^2$, (5) urea x year interaction, (6) pounds of corn silage fed, (7) urea from corn silige, (8) $Urea^3$, (9) $Urea^4$, (10) urea from dry grain, (11) number of cows and (12) urea from commercial concentrate supplements.

All of the above variables had partial correlation coefficients with adjusted calving interval less than R = .05 and were nonsignificant ($P > .1$).

The variables retained in the final equation were: year, months corn silage was fed, percent of herd bred by artificial insemination, days from calving to first breeding, and milk production per cow. Each of these variables were partially correlated with adjusted calving interval (r = .05) and were statistically significant different from zero ($P < .003$ to .0005). Since only about 15 percent of the total variation in adjusted calving interval was accounted for by all the variables and all of these were weakly correlated with calving interval it is concluded that none of these were sufficiently important to have practical value in determining the length of the calving interval.

Relationship Between Levels of Urea and Percent Cows Sold for Sterility:

To further test whether feeding urea affects reproduction, data were compiled from herds in this study concerning the relationship of urea use and the percent of cows sold because of sterility. Table 2 shows within each of the levels of urea fed the percent of cows sold for sterility. The mean percent of cows sold for sterility in herds fed no urea (2.15%) is significantly different than the mean in herds fed no urea (2.42). However, the difference is only .27%, and of no practical significance. This would represent only one more cow in 400 being sold for sterility in herds fed urea. The 95 percent confidence interval is 0.27 percent ± the mean.

Thus, the probability is 95 percent that the mean percent cows sold for sterility is between 2.02 and 2.52 percent, and it is very unlikely that more than 0.27 percent more cows would be sold for sterility if fed urea. It should be noted from the data in Table 2 that the mean percent sold for sterility was lowest (1.71 percent) in the group of herds fed the highest level of urea. These data concur with the findings of Patton et al. (83), Miller et al. (71) and Holter et al. (49) that the feeding of urea did not affect fertility of dairy cattle.

Dairymen's Knowledge and Attitudes About Urea

A portion of the questionnaire was devoted to determining the dairyman's attitude and understanding about urea: whether he thought it caused breeding difficulties; if he had discontinued its use and fome indication as to his reason for terminating his use of urea. It was obvious from the section of the questionnaire that dealt with the type and urea status of the protein supplements used by dairymen that many dairymen did not know that their commercial protein supplements contained urea. Over the five-year survey period, 631 dairymen responded to this question. Of this number, 42 dairymen answered that they knew that their protein supplement contained urea, 27 dairymen answered incorrectly and 562 dairymen did not indicate whether or not their protein supplement contained urea. There seemed to be no question in the minds of the respondent dairymen whether they added urea to corn silage, high moisture grain or dry grain, as evidenced by their answers to questions in these areas. Thus, it may be assumed that a good portion of the dairymen who did not indicate the urea status of their protein supplements responded in this manner because either they did not know or were not sure if they contained urea.

Another attitude question related to whether the dairymen thought urea and herd breeding difficulties were in any way connected. Only 45 dairymen definitely thought there was a relationship. Five hundred fifty-eight dairymen did not respond to this question, and 27 others indicated urea had no connection with breeding difficulties. It seems evident from this question that the bulk of the dairymen included in this study did not relate breeding difficulties to urea fed in dairy cattle diets.

Many dairymen in this study did not respond favorably to urea use in dairy cattle rations. Twenty-two percent of the dairymen in this study definitely indicated that they did not favor feeding urea. Only six percent responded favorably, while 72 percent did not indicate their preference. It would appear from the response to this question that a considerable number of the dairymen in this study have some reservation about urea and its use.

Sixty-five percent of the 96 dairymen in this study who quit using urea during the five-year period did so for reasons of ration palatability or their personal dislike of urea products. Twenty-four dairymen or 24 percent of those who quit did so because they thought urea caused reproductive difficulties.

It is apparent that there are many conflicting ideas and attitudes about urea, its use and its overall effect upon dairy herd performance among the dairymen included in this study.

SUMMARY AND CONCLUSIONS

Data from Michigan Dairy Herd Improvement Association (DHIA) herds was studied to determine if there was a relationship between the feeding of urea and the reproductive efficiency of dairy cattle.

A total of 3157 herd year observations representing 85,281 calving intervals were calculated from DHIA records for the five-year period 1965-1969. Data concerning feeding practices furnished by the dairymen through a survey form were used to calculate the average amount of urea fed per cow daily for each of the herds. The average total amount of urea fed daily in herds fed urea was 80.6 grams. The average amount of urea fed per cow daily from various sources was: urea-treated corn silage 40.0 grams, commercial protein supplements 32.6 grams. Urea additions to dry grain rations provided 7.5 grams and urea treated high moisture corn (grain), 0.6 grams.

Adjusted calving interval (ACI), computed as calving interval minus days from calving to first breeding as herd policy, and percent of cows sold for sterility from DHIA records were used as the measures of reproductive efficiency.

Correlation analysis of the ACI and total grams urea fed per cow daily resulted in a simple correlation $r = .03$, which was non-significant. Partial correlations between each of the sources of urea and ACI were of the order $r = .02$ to $.01$ and non-significant. Similarly the number of years urea was fed was not related to length of calving interval as indicated by low correlation ($4 = .01$) for year by urea interaction and adjusted calving interval.

The average length of calving interval was 379.9 days for herds fed urea and 380.4 days for herds fed no urea.

From the statistical analysis of the data it was concluded that neither the level of urea fed, nor the number of years urea was fed affected reproductive efficiency in dairy cattle in Michigan DHIA herds. Average calving interval was 5 to 6 days longer for the years 1967 and 1969 than for the other three years studied whether or not urea was fed. Reasons for the lower reproductive efficiency some years than others were not revealed by this study.

These findings agree with the results of research reported in the literature where cow numbers were much smaller.

Table 1. Mean, range and standard deviation of variables studied in relation to length of calving interval from 3157 observations of Michigan DHIA Herd Records, 1965 through 1969. Data include 1709 observations in Herds Fed Urea.

	Mean All herds	Min.	Max.	S.D.	Mean urea intake for herds fed urea
Number of cows/herd	48.6	1	1247	47	
Milk/cow/305 day (lb)	12964.0	5506	20074	2195	
Adjusted calving interval (d)	315.5	244	496	23	
Calving to 1st breeding (days)	66.2		99	12	
Percent bred A.I. (av)	74.2		100	35	
Corn silage fed/cow/day (lb)	38.4	-	-	-	
Months corn silage fed (mo)	7.5	-	-	-	
Grain fed/cow/day (lb)	12.3	-	-	-	
Urea from corn silage (g/day)	21.6		231	42.1	39.9
Urea from HM corn (g/day)	0.3		78	3.6	0.6
Urea from dry grain (g/day)	4.1		177	18.8	7.5
Urea from commercial supplement (g/day)	17.7		224	27.6	32.6
Urea total intake (g/day)	43.3		370	55.5	80.6

Table 2. Average calving interval, percent cows sold as sterile, and milk production of herds fed various levels of urea compared to herds fed no urea. 1965-69 Michigan DHIA.

Urea Levels (g)	None	1-370	1-60	61-120	121-180	181+
Average urea (g)	0	79.8	36.1	90.5	146.7	219.5
No. herd - year obs.	1442	1715	760	653	219	83
Adj. Calving Int.* (d)	314.4	315.7	313.4	317.8	316.5	313.7
Days adjusted	67.3	65.2	66.7	64.4	63.3	64.2
Calving inter.* (d)	380.4	379.9	379.4	380.7	379.8	377.8
Sold Steril. (%)	2.15	2.4	2.4	2.4	2.6	1.71
No. Cows with no CI av.	21.2	22.1	21.6	22.7	22.9	21.2
No. Cows av.	42.3	53.8	45.6	60.6	59.3	60.7
No. Cows CI av.	23.9	29.6	25.5	33.3	32.1	32.4
Milk per cow, lb.	13046	12895	12747	12952	13146	13136

$$\text{*Weighted CI and Adj. CI} = \frac{\text{Total No. CI days}}{\text{Total No. CI cow observations}}$$

Table 3. Mean adjusted calving interval by years for herds fed no urea and urea (all levels).

Year	Herds fed no urea		Herds fed urea			Total No. herds
	No.	ACI	No.	ACI	% of herds	
		av. days		av. days		
1965	306	312	229	312	42.8	535
1966	315	314	321	313	50.5	636
1967	280	318	341	319	54.9	621
1968	272	312	351	313	56.3	623
1969	275	316	348	318	55.8	623

ACI = adjusted calving interval (days)

Table 4. Simple correlations among variables studied in relation to calving interval and urea feeding.

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	
1. Year	1.00																		
2. Grain	.09	1.00																	
3. Corn sil.	.03	.00	1.00																
4. Mo. corn sil.	.04	.06	.02	1.00															
5. Vit. A.	.00	.10	.08	.09	1.00														
6. Urea C.S.	.17	.06	.30	.15	.06	1.00													
7. U HM corn	.03	.07	.06	.07	.01	.12	1.00												
8. U dry cr.	.03	.04	.02	.02	-.06	.10	-.02	1.00											
9. U C C Sup.	.03	.05	.01	-.01	.09	.02	-.02	.02	1.00										
10. Tot urea	.04	.09	.24	.21	.07	.79	.14	.43	.49	1.00									
11. A.I.	.01	-.02	.00	.01	.01	.04	.03	.00	-.01	.03	1.00								
12. days delay	-.02	-.03	-.08	.00	-.01	-.12	-.05	-.05	-.02	-.12	-.06	1.00							
13. No. cows	.07	.07	.19	.16	.07	.12	.02	.02	.08	.14	.00	-.11	1.00						
14. # milk	.00	.19	.01	.09	.05	.02	.03	.03	.04	.01	.11	.05	-.02	1.00					
15. ACI	.06	.01	.03	.05	.01	.05	.04	.00	-.02	.03	.08	-.37	.08	-.07	1.00				
16. Y x U	.15	.09	.24	.12	.07	.79	.14	.42	-.49	.99	.03	-.12	.14	.01	.03	1.00			
17. U^2	.11	.10	.21	.09	.03	.70	.13	.52	-.36	.90	.02	-.09	.10	.01	.02	.90	1.00		
18. U^3	.08	.08	.14	.05	.01	.52	.09	.54	.27	.72	.00	-.07	.05	.00	.01	.72	.94	1.00	
19. U^4	.05	.06	.08	.02	-.03	.37	.05	.52	.20	.56	-.01	-.05	.03	-.01	.00	.56	.83	.97	1.00

Legend

1. Year (1965-69)
2. Grain, amount fed
3. Corn silage, amount fed
4. Months Corn silage was fed
5. Vitamin A supplement fed
6. Urea from corn silage (grams/head/day)
7. Urea from high moisture corn (g/h/d)
8. Urea from dry concentrate mix (g/h/d)
9. Urea from commercial supplement (g/h/d)
10. Total grams urea fed per head per day.
11. Percent of cows in herd bred by artificial insemination
12. Days intentional delay from calving to first breeding
13. Number cows in herd
14. Pounds milk per cow (herd average)
15. Adjusted Calving Interval
16. Y x U = year x urea level interaction
17. U^2 = Urea levels

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