DAIRY CATTLE BREEDING PERFORMANCE WHEN GRAZING THE HIGH PROTEIN PASTURES OF URUGUAY

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Hugo Ricardo Tosi

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Abstract

Management and breeding information of 602 dairy cows and 3696 dairy heifers was obtained from the 1993, 1994 and 1995 records of four farms to study the dairy cattle breeding performance under Uruguayan commercial farming conditions. When diet was based on an unsupplemented legume pasture first service conception rates of lactating cows were 43.2 and 50.3%, services per conception 2.20 and 2.25 and overall pregnancy rates (% of bred) 82 and 88% on two dairy farms, respectively. Lactating cows, from one farm, fed legume pasture supplemented with corn silage and cereal grains showed better performance with first service conception rate being 62.7%, services per conception 1.63 and overall pregnancy rate (% of bred) 90.1%. Dairy heifers grazing legume pastures had better performances than lactating cows grazing legume pasture, but the response was variable among seasons and years. First service conceptions ranged from 63.5 to 75.1, services per conception from 1.27 to 1.81 and overall pregnancy rates from 78.6 to 96.1. Although many factors could have determined the differences observed a dietary effect related to the high CP content of legume pastures was not dismissed. Based on the results obtained from the farm survey two grazing trials were conducted to evaluate the breeding performance of nulliparous Holstein heifers under three dietary treatments with different levels of crude protein. Heifers grazed a red clover (Tri*folium Pratense)* pasture as the basic component of the diets. One diet consisted of pasture alone while for the other two corn silage was utilized to supplement pasture and to reduce CP intake. Diet did not affect the breeding performance of heifers as measured by pregnancy rate (% of bred) during the two breeding seasons evaluated (winter and spring) and after two chances of AI.

Supplementation with corn silage at 2.0% of BW (DM basis) resulted in a lower proportion of heifers observed in estrus (60.1 % vs. 82.6%) and in a lower first service pregnancy rate (50.0% vs 71.4%) during spring. Reduced SUN and increased progesterone levels during the periovulatory phase on heifers fed the high silage diet may have determined the differences observed. It is concluded that high CP content of legume pastures did not affect the breeding performance of Holstein heifers and that supplementation of legume pastures with moderate amounts of silage resulted in heifers with similar chances to become pregnant as herdmates fed on pasture alone. However, when pastures are supplemented with large amounts of silage special strategies for estrus detection should be considered.

Dedicated to my wife Silvia, and my children Rafael, Martin, Alejandro and Romina,

for their love and support

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ABBREVIATIONS

ADF = acid detergent fiber

ADG= average daily gain

AI= artificial insemination

BCS = body condition score

BUN= blood urea nitrogen

BW = body weight

° C= degree Celsius

Ca = Calcium

cAMP = cyclic adenosine mono-phosphate

CARECO = Campo de Recria Colonia (Colonia Co-operative Rearing Farm)

CL = corpus luteum

CONAPROLE= Cooperativa Nacional de Productores de Leche (National Dairy Farmers Co-operative)

CP = crude protein

CPDM= crude protein in feed dry matter

CR= conception rate

Co = cobalt

Cu = copper

DCP = degradable crude protein

DFO= days to first ovulation

dl = decilitre

DM = dry matter

DMI = dry matter intake

Fe = iron

FSH = follicle stimulating hormone

GH = growth hormone

GLM = general linear model

ha = hectare

h = hour

IU= international unit

INIA = Instituto Nacional de Investigacion Agropecuaria (National Institute of Agricultural Research)
kg = kilogram

LH = luteinizing hormone

LHRH= LH releasing hormone

LSM = least square mean

NDF = neutral detergent fiber

Mcal = Megacalories

Mg = magnesium

mg = milligram

Mn = Manganese

Mo = molybdenum

N= nitrogen

Neg = net energy of gain

Nel = net energy of lactation

Nem = net energy of maintenance

P= phosphorus

PG = prostaglandin F2a

ppm = parts per million

PUN= plasma urea nitrogen

RUP = rumen undegradable protein

S = sulphur

Se= selenium

SEM = standard error of the mean

SUN = serum urea nitrogen

t = metric tonne

UIP= undegradable intake protein

WSC = water soluble carbohydrates

Zn= Zinc

Introduction

Dairy industry in Uruguay have been steadily growing during the last two decades. Since dairy production is based on a year round grazing system, the increase in milk production is directly related to an increase in the use of cultivated pastures. The short life of perennial species under Uruguayan grazing conditions have led to the implementation of a variety of rotational schemes by alternating legume pastures with annual forage crops. The inclusion of legumes pastures in a rotational scheme have the dual purpose of keeping the physicochemical properties of the soils and providing a good quality feed supply (Diaz et al., 1980).The most commonly utilized species are red clover *(Trifolium Pratense L.)* white clover *(Trifolium repens)* and birdsfoot trefoil *(Lotus corniculatus L.)* (Diaz et al. 1996).

In order to adapt milk production to the market needs and to take advantage of the better milk yields obtained with fall-winter calving (Faggi et al, 1978), breeding usually takes place from May to January. A variety of feeding strategies are utilized during the service periods. Farms managed at low stocking rates usually overcome the pasture shortages occurring during winter (June to August) by supplementing grazed legume pastures and forage crops with pasture hay. Farms managed at high stocking rates normally supplement the same type of grazed pastures with corn silage and concentrates. During the spring months (September to December) legume pastures are usually the only component of dairy cows diets in most farms. Dairy heifers when reared on the farm are usually confined to low quality pastures, determining low rates of gain and delayed puberty. Collective heifer rearing farms were developed during the last 10 years in an attempt to increase the grazing areas for lactating cows on the farm and to improve heifer development. On collective farms high quality legume pastures are usually utilised to accomplish better growth performance of the dairy heifers.

Reproductive performance under the described circumstances is generally assumed to be acceptable. However, season to season and year to year variations have been identified at farm level creating the concern in many dairy producers that poor performances observed during certain breeding seasons may be associated with the diet. The scarce documentation available shows reduced performances during spring services relative to winter services at the experimental herd at La Estanzuela Experiment Station. Data described by Cavestany (1992) indicate that during 1990 pregnancy rates of the experimental herd at La Estanzuela were 83 % in winter and 70 % in spring while in 1991 they were 62 % in winter and 40 % in spring. In this case the dependence on high crude protein pastures as the sole component of the diet in spring has been described as one possible reason for the poor fertility observed. (Cavestany, 1992).

Many studies have investigated the effect that high CP diets may have on the reproductive efficiency of high producing dairy herds due to the trend to overfeed protein during peak lactation in many north American dairy herds. Most of these studies have reported a negative relationship between fertility and dietary CP (Claypool et al., 1980; Edwards et al., 1980; Ferguson et al. 1988; Ferguson and Chalupa, 1989; Ferguson et al. 1993), but some others (Howard et al. 1987; Barton et. al. 1996) did not find any association or identified other effects such as uterine health interacting with CP and confounding its effect on fertility.

Under Uruguayan management practices, some of the usual service periods are coincident with the utilization of legume pastures as the only component of the diet of cows and heifers. The species utilized are reported to have CP contents above 17% when whole plants are considered (NRC, 1989; Cozzolino et al. 1994). Furthermore, the protein content of the consumed pasture can easily exceed those levels when low grazing pressures are allowed.

There is no experimental evidence indicating an effect of CP on the reproductive performance of dairy cows or heifers in Uruguay. However, it may be that the high protein content of the legume pastures are playing a role on the occasional reductions on the fertility of dairy herds fertility causing concern for people involved in milk production.

The objectives of this study are: 1) to asses the actual incidence of the problem and the degree of variation among service periods by collecting reproductive and feeding management information from commercial farms, and 2) to evaluate the breeding performance of dairy cattle grazing legume pastures as compared to alternative feeding strategies with reduced CP content.

Literature review

FORAGE PRODUCTION IN URUGUAY

Uruguay is situated within 30° to 35° south latitudes. Its proximity to the sea and its location in the hemisphere were the proportion of water to land is 15:1 determines milder temperatures than that observed in similar latitudes in the northern hemisphere were the proportion is 5:1 (Corsi, 1978). Average temperatures south to north of the country range from 11° to 14 ° C in July (coldest month), and from 22° to 27° C in January (warmest month), respectively. Winter frosts occur every year in central Uruguay and 76 to 84 % of the years in the rest of the country. Average rainfall is 930 mm in the south and 1300 mm in the north (Corsi, 1978).

More than 70% of the 17,600,000 hectares of Uruguayan land is dedicated to forage production (Allegri and Formoso, 1978; Mas, 1978; Symonds and Salaberry, 1978; Termezana, 1978). Native pastures constitute 85% of the total forage producing area, and compose the main forage resource for extensive, low input animal production (Carambula et. al. 1997). Intensive animal production with high stocking rates is supported by cultivated pastures and fodder crops in the remaining 15% of forage lands and including the better quality soils of Uruguay.

Climate is advantageous for pasture development and grazing on a year round basis. However, pastures grow more actively during spring and fall than in summer and winter. Restrictions to plant growth are determined by limited water availability in the soil in summer, and by lower temperatures and poorer solar radiation in winter (Corsi, 1978). Soil variability and grazing management are also significant contributions to the variation in seasonal pasture growth, dominant species, stand density and stand productivity.

NATIVE PASTURE LANDS IN URUGUAY

Native pasture lands used for animal production in Uruguay consist of grasses, forbes and shrubs with few trees (Berreta and do Nascimento, 1991). The species composition of native pastures is complex and dynamic, and the relative frequency of species changes. In a given soil group, animal grazing management and season determine a great deal of those changes. Millot (1991) describes two commonly used strategies for grazing in native pasture lands in Uruguay.

Stategies consisting of continuous grazing with high stocking rates results in the establishment of a group of creeping grass species tolerant to grazing. Some of the predominant species include *Paspalum notatum*, *Paspalum distichum*, *Paspalum nicorae*, *Axonopus affinis*, *Pennisetum clandestinum*, *Bouteloua megapotamica*, *Cynodon dactyon* and *Eleusine tristachya*. These species belong to the C4 photosynthetic group and therefore the majority of dry matter (DM) yield occurs in the spring and summer seasons. Warm season grass pastures will include small weeds and bare soil areas.

A second strategy is continuous grazing with low stocking rates allowing animals to selectively consume forages. This strategy not only results in the presence of the creeping warm season grasses, but also in a higher layer of umpalatable forage biomass. Predominant summer species in this upper layer are *Andropogon lateralis*, *Elyonorus spp.*, *Erianthus trinii*, *Sorgastrum pellitum*, *Sporobolus indicus*, *Paspalumn quadrifarium*, while winter ones include *Stipa brachychaeta*, *Stipa charruana*, and *Aristida spp.* Together with these grasses a significant amount of weeds and shrubs establish in the upper layer. Some of the dominant weeds include *Acacia spp.*

Baccharis spp Eupatorium spp. Vernonia spp. Eryngium spp. and *Solidago chilensis.* This upper forb canopy provides a very low contribution to the animal DM intake but will protect the valuable, tender winter species from grazing. The C3 photosynthetic group making up these winter species include *Bromus auleticus, Calamagrostis spp. Briza spp. Piptochaetium spp. Stipa spp* and summer species like *Coelorhachis selloana, Paspalum dilatatum, Paspalum urvillei. Paspalum plicatulum,* and *Setaria spp.* This protected pool of seeds has been identified as an important component of the pasture that can respond to changes in cattle management and improvement in pasture quality.

Native legumes are scarce and appear in some areas mainly in spring. *Trifolium Polymorphum* has been identified as the most relevant native legume in Uruguay (Formoso 1990). *Medicago polymorpha, Adesmia spp., Vicia spp., Rynchosia, Arachis spp.* also have been identified in a wide range of soils with variable frequency (Allegri and Formoso, 1978; Risso and Scavino, 1978; Symonds and Salaberry, 1978; Temezana, 1978).

Seasonal changes in the cover structure have been described by Berreta (1996), who observed increases in cover density and in the amount of good quality grasses and reductions of forbs and fibrous grasses from winter to spring.

Variable productivity has been observed in different soil types. Annual DM per hectare is 2500 kg or less for low fertility shallow soils, 3500 kg for medium depth soils and 5000 kg or more in deep fertile soils, and deep sandy soils with large capacity for water storage. (Zunino and Batista, 1988; Formoso, 1990; Berretta 1991; Berretta and Bemhaja, 1991).

Average seasonal DM distribution for a wide range of soils is 25% (± 10) , 29% (± 6), 16% (± 3) and 30% (± 4) for summer, fall, winter and spring respectively (De Souza, 1985).

Average crude protein content is 8.4 (± 0.6), 9.5 (± 0.7), 12.5 (± 3.2), and 8.3 (± 1.0) for summer, fall, winter and spring, respectively (De Souza, 1985). According to Cozzolino et. al. (1994), native pasture annual average organic matter digestibility is 55.3 %, crude protein content is 11.7 % and net energy of lactation of native pasture is 1.1 Mcal per kg DM. Phosphorus (P) content follows the same seasonal pattern as protein. Summer species P content range from 0.08 to 0.11 %, DM basis and winter species from 0.2 to 0.3 %, DM basis. Phosphorus shortages for animal production are expected to occur on spring-summer native pastures(Berreta 1996). Calcium content of native grasses varies from 0.5 to 0.7 % DM basis (Berreta 1996), which is borderline to meet the requirements for young growing dairy cattle and enough for a moderate milk production in the case of dairy cows as determined by NRC (1989). Traditional grazing strategies on native pastures involved mixed species grazing with a combination of sheep for wool production and cattle for beef production. Uruguay's mean ratio was 2.3 sheep per bovine at a stocking rate of 0.82 "cattle units" [1] per hectare (Risso and Scavino 1978).

The dairy industry developed on medium-good quality soils in the area surrounding Montevideo, capital city of Uruguay. Until 1970, native pastures played an important role as a major component of the diet for dairy cows. Supplementation with cheap concentrates like wheat bran and establishment of small acreage with winter fodder crops like oats were utilized as well. During the last twenty to thirty years an intensification and expansion process occurred in the dairy industry. Native pasture lands were slowly replaced by cultivated pastures that include high quality, productive legume species. The utilization of winter and summer annual crops, inclusion of energy

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[1] cattle unit is equivalent to maintenance requirements of an open and non lactating beef cow weighing 450 kg. (Rivera and Carrau 1994).

and protein concentrates and forage conservation procedures were widely adopted as well. Despite these advances most farms still have native pasture acreage which is used for dry cow and heifer feeding.

CULTIVATED PASTURE LANDS IN URUGUAY

Dominant species

García (1996a) describes three kind of cultivated pastures in Uruguay: annual pastures, short rotation pastures and long rotation pastures.

The most common species in annual winter pastures include: oats *(Avena byzantina C. Koch)*, ryegrass *(Lolium multiflorum L.)*, wheat *(Triticum aestivum L.)*, barley *(Hordeum vulgare L.)*. Warm seasonal crops such as: Sudangrass *(Sorghum sudanense Piper Stapf)*, Sorghum (*Sorghum bicolor*)-Sudangrass hybrids and Corn (*Zea Mays*) (García 1996a) are more common on summer pasture. Annual crops may be used for grazing, silage, or for double purpose grazing-silage or grazing-grain production. Wheat, barley and oats are also utilized as nurse crops when establishing perennial pastures.

Red clover (*Trifolium pratense L.*), alone or in mixtures with ryegrass and oats constitute a common example of a high yielding two year (short rotation) pasture (García 1996a).

Long rotation pastures include different combinations of perenial legumes and grasses (García 1996a). White Clover (*Trifoliumrepens*), red clover (*Trifolium pratense L.*), birdsfoot trefoil (*Lotus corniculatus L.*) and alfalfa (*Medicago sativa L.*) are the most commonly used legumes (Diaz et al. 1996), while italian ryegrass (*Lolium multiflorum L.*), tall fescue (*Festuca* *arundinacea Scherb.*) and phalaris (*Phalaris aquatica*) are mentioned by Rebuffo and García (1991) as the more relevant grasses in long term pasture establishment.

Several varieties of these species, either obtained by Uruguayan plant breeding work or introduced material from other sources, are currently available for Uruguayan farmers. Some of the more important varieties described by Rebuffo and Garcia (1991) and Garcia (1996b) are: Zapicán, Bayucuá and Regal white clover; LE 116, El Sureño and Kenland red clover; E. Chaná and Crioula Alfalfa; San Gabriel and Ganador birdsfoot trefoil; LE 284 and Matador ryegrass; Tacuabé, El Palenque and Dovey tall fescue; and Urunday and El Gaucho phalaris.

Seasonal dry matter yield and quality

Garcia (1996a) summarizes DM yield data of several cultivated pastures. Ryegrass and oats DM production is restricted to fall, winter and spring, yielding 7.0 and 4.5 tonnes of DM per hectare per production cycle, respectively. Wheat production is restricted to the winter season and DM production under grazing conditions is 2.0 tonnes per hectare. Grazed sorghum, sudangrass and corn supply DM in the summer and fall months. Annual dry matter production is 9.0, 13.0 and 7.5 tonnes per hectare for sudangrass, sorghum and corn respectively.

A mixture of oats, ryegrass and red clover in short rotation pasture will produce 9.9 and 7.4 tonnes DM during the first and second years, respectively (Garcia 1996a).

Carambula (1978), summarizes many experiments that compared DM yields of native pastures with long rotation pastures on different soil types. Dry matter yields for cultivated pastures were 100 to 566 % higher than for native pastures. However, pasture stand life is quite short in cultivated pastures, with peak DM production during the second year and a significant decrease during the third year. Mean fourth year yields are even lower with a high coefficient of variation

(García et. al. 1981). Garcia (1996a) describes the DM yield of a tall fescue, white clover, birdsfoot trefoil pasture as 6.0, 9.0, 6.0 and 5.5 tonnes per hectare for the first, second, third and fourth years, respectively.

Long rotation pastures are usually a combination of grasses and legume species however, legumes are the dominant component (García 1996a). It was observed that third year DM yield decrease is associated with a significant decrease in the proportion of legume plants in the pasture (García et. al. 1981).

Diaz et. al. (1996) studied the information on legume productivity of four legume species from 33 experiments. Second year DM yields were: 11.6 t for alfalfa, 8.8 t for red clover, and 7.5 t for white clover and birdsfoot trefoil. During the third year red clover disappears from the stand, alfalfa produces 8.5 t, white clover 2.8 t and birdsfoot trefoil 6.3 t DM.

Seasonal dry matter production follows a similar pattern as for native pastures, but with a more significant contribution during the spring. The experiments reviewed by Diaz et. al. (1996) reveal that birdsfoot trefoil and red clover produced 50% of the annual DM yield in the spring with the remaining 50% equally distributed during the summer, fall, and winter; white clover also produced 50% in spring but its fall-winter production was much larger than in summer; and alfalfa produced almost 50% in summer months and only 17 % in fall-winter period.

Many factors contribute to the low persistence of the improved pastures in Uruguay, the more significant ones being: soil compaction, presence of weeds such as bermuda grass (*cynodon dactylon*), the low soil P availability, seeding methods, and grazing management (García et. al. 1981).

High DM digestibility have been observed for cultivated forage on winter pastures with lower digestibility for summer pasture, however the grass:legume ratio and pasture management can affect pasture quality (Leborgne 1984). For alfalfa, red clover, birdsfoot trefoil, ryegrass, tall fescue, phalaris, oats, wheat and sorghum pastures in vegetative stage respectively, organic matter digestibility (%) is 70.3, 63.2, 60.3, 70.1, 63.4, 73.5, 77.0, 77.9 and 61.5, crude protein content (%) of DM is 20.3, 15.6, 17.2, 14.6, 12.7, 14.1, 14.4 , 17.6, 19.6, 10.4 and net energy of lactation as estimated from acid detergent fiber content, is 1.4, 1.5, 1.1, 1.3, 1.2, 1.3, 1.5, 1.4 and 1.4 Mcal per kg of DM (Cozzolino et al. 1994).

Historical and current use of cultivated pasture lands

Perennial grasses and legumes for livestock grazing, were originally established in order to create a more sustainable farming system by alternating cereal production with animal production.. These pastures have the dual purpose of improving the physicochemical properties of the soils and providing a good quality feed supply (Diaz et. al. 1980).

The first cultivated long rotation pastures were established in the early sixties. Commercial seeds for these pastures were imported mainly from New Zealand. However, the first species had poor performances in terms of yield and longevity as compared with cultivars evaluated and developed more recently as a result of local plant breeding work (Garcia et. al. 1981, Garcia 1996b). The better adaptation of locally evaluated cultivars has resulted in a steady expansion of the area of improved pastures since 1970's.

Cultivated pastures are currently utilized in the south-west of the country, where the best soils occur, and provide the basic diet in intensive farming systems like dairy and beef cattle fattening.

Intensive beef production is usually associated with relatively simple pasture-cereal crop rotation systems. Concentrate supplementation silage and hay production do not play an important role in beef production.

A more complex situation is observed in dairy production systems where forage rotation systems are widely utilized. The short life of the cultivated pastures, and seasonal variations in pasture DM yield require that carefully planned pasture rotations be adopted. A basic restriction is that the area utilized for long term pastures has to be less than 60% of the total farm acreage, since the pastures have to be replaced approximately every four years (Duran 1992). Several possibilities arise by combining species of different cycle and production potential. The combination utilized will determine the productive levels and the seasonal DM production.

Since animal diets are basically derived directly from grazing, the efficiency of production is assessed as milk production per hectare. Duran (1992) mentions that no more than 3,000 l of milk per year per hectare can be achieved with a simple rotational system such as a three year pasture combined with one summer forage crop and two winter forage crops. However, 6000 to 7000 l per hectare can be obtained with more complex rotations, the use of concentrates, and grass/legume or corn silages (Duran 1992).

DAIRY CATTLE FEEDING AND MANAGEMENT

Dairy farms in Uruguay produce milk on a year round basis, however, the continuous calving system commonly adopted a few years ago is being replaced. The current breeding program is manipulated in accordance with the market needs and seasonal forage production. Therefore, breeding usually takes place from May to January, with one peak in the fall-winter months May-July and another one in the spring-summer months November-January.

Usually there is no breeding from February to May in order to avoid a summer calving, when pasture quality is poor for cows in early lactation. Furthermore, a study conducted by Faggi et. al (1978) comparing lactation curves of cows calving through out the year have shown that the largest milk productions are obtained with cows calving in fall and winter as compared to spring and summer, with cows calving in May and June producing the largest amounts of fat corrected milk.

The pasture rotation scheme used by each farm is adapted to soil type, land and herd size, machinery availability etc. However, on most farms the feeding strategy of dairy cows follows a similar pattern. Long and short rotation pastures are usually the sole component of lactating cow diets in the spring. Cows are fed a combination of annual, short and long rotation pastures together with silage, hay and concentrates during fall and winter. Summer feeding is usually restricted to annual summer pastures and supplementation with a grass/legume silage.

Farms managed with low stocking rates have less defined calving peaks and milk production follows the seasonality of pasture DM production. Maximum production of milk is usually achieved in the spring months, September to December, when pastures reach the maximum rate of DM production. Grass/legume hay and low amounts of concentrates commonly are used as supplements for the winter pasture. Summer feeding is restricted to forage crops with no supplementation.

On high stocking rate or more intensively managed farms, the two calving peaks (fall and spring) are usually well defined. High levels of milk production are achieved during the winter when the export market demand is high and better prices are obtained. In that scheme winter pasture shortages are overcome with corn silage and concentrates. Spring pasture excesses conserved as silage together with a limited amount of concentrates are used to supplement summer forage crops.

Replacement heifers are usually confined to areas with poor quality pasture, as the best areas are reserved for the lactating cows. Concentrate supplementation of heifers diets is not common. Therefore, relatively poor rates of growth are observed resulting in heifer age at first service averaging 24 months.

The use of cultivated pastures has contributed to improve the nutritional status of Uruguayan dairy herds. However, there is concern by many dairy producers that the poor reproductive performances observed during certain breeding seasons, may be due to problems with the diet.

Cavestany (1993) described the 1990 and 1991breeding performances of the herd at La Estanzuela Experiment Station (222 cows in 1990, 226 cows in 1991). Cow pregnancy rates, as a percentage of total cows, were 82.9% in winter and 69.8% in spring during 1990, and 62.5% in winter and 40.0% in spring during 1991. Breeding performance of dairy heifers at the same Experiment Station was also described by Cavestany (1992). A pool of breeding data from 1991 and 1992 of 60 heifers indicate pregnancy rates, as percentage of total heifers, of 68% in winter and 40% in spring. Qualitative aspects of local feeding strategies were pointed out as a possible restriction to reproductive performance. A high crude protein (CP) content in cultivated pastures, and the dependence on pasture as the sole component of the diet in spring have been implicated as one reason for poor breeding performance during the spring season (Cavestany 1992).

THE ESTROUS CYCLE

Butler (1998) described conception and the establishement of pregnancy as an ordered progression of events involving all the various tissues of the reproductive tract: follicular development resulting in ovulation, fertilization of the oocyte, embryo transport and development, maternal recognition and implantation. Follicles develop in the ovary in a wave-like pattern with the growth of a number of small follicles followed by selection of a dominant follicle (Ginther et al. 1989). Two or three waves of follicular growth occur during the estrous cycle of the cow (Ginther et. al 1989). Follicular maturation is induced by gonadotropins released from the pituitary, the two most important gonadotropins are follicle stimulating hormone (FSH) and luteinizing hormone (LH). Under the influence of pituitary gonadotropins the follicle secretes estrogens and plasma concentrations of the primary estrogen, estradiol, reach peak levels (Schams et al. 1977, Kaneko et al. 1991). Estrus behaviour is induced by estradiol in the relative absence of progesterone (Lemon et al. 1975). Estradiol is also invloved in a discharge of LH from the pituitary gland that occurs simultaneously to the estrus period (Lemon et al. 1975) leading to ovulation. Once ovulation has occurred, a corpus luteum develops in the ovary which secretes progesterone (Allrich 1994). Progesterone is needed to maintain pregnancy, however, if there is no fertilization or the subsequent steps to establish pregnancy fail to take place, prostaglandins secreted by the uterus lead to the lysis of this corpus luteum and a subsequent decrease in progesterone levels allowing the growth of new follicles (Quirk et al. 1986). Summarized data presented by Schams et al. (1977) indicated that the period of time between two ovulations (estrous cycle) is 21 days in the cow.

MANAGEMENT AND FEEDING FACTORS THAT AFFECT REPRODUCTIVE PERFORMANCE IN DAIRY CATTLE

Estrus detection and syncronisation

Accurate estrus detection and proper timing of insemination remain as major problems limiting breeding performance of dairy herds (Larson and Ball, 1992). Fifty percent of the estrus periods are usually missed in dairy herds (Stevenson and Britt, 1977). Different methods to regulate the estrous cycle have been developed to control the time of estrus and ovulation in order to inseminate cows during the desired post-partum period to improve the reproductive performance (Larson and Ball, 1992). The initial attempts to regulate estrus cycle involved administration of exogenous progesterone, or synthetic progestogens to prolong the luteal phase of the estrous cycle (Patterson et al. 1989). Stage of the cycle at which the treatment is initiated and the length of the treatment are two major factors affecting the effectiveness of estrus synchronisation methods using progestogens alone. (Gyawu, and Pope, 1983; Patterson et al,. 1989). The addition of luteolytic agents as prostaglandin to progestogen treatments improves the efficiency of synchrony and fertility (Gyawu, and Pope, 1983). Luteolytic agents alone are also administrated to synchronise estrus. Cavestany et al. (1988) reported that exogenous administration of luteolytic agents mimics the natural initiation of luteolysis in the cycling animal. Prostaglandin $F_{2\alpha}$ and analogues as cloprostenol, are described by Larson and Ball (1992) as the most effective luteolyitic agents for synchronising estrus in cattle. However, the response concerning the time at which onset of estrus occurr in relation to prostaglandin injection is inconsistent. The inconsistent response has been related to season (Britt et al. 1978), age (Burfening et al. 1978), breed (Moore 1975) and the functional status of the corpus luteum preceding induced ovulation (Richards et al. 1990). Ryan et al. (1995) found a 60.9 % pregnancy rate in cows inseminated at a fixed time 3 days after prostaglandin injection. The administration of other hormones like human chorionic gondotropin combined with estradiol and gonadotropin releasing hormone in combination with prostaglandin were attempted to overcome inconsistencies on the response to prostaglandin alone (Lopes Gatius ,1989; Archbald et al. 1992). These treatments improved the accuracy of the predicted time at which estrus may occur in response to prostaglandin but increased the cost. Prostaglandin treatment followed by estrus observations have been evaluated resulting in 40 % less services per conception than a combination of a fixed-time insemination and estrus observation schedule after prostaglandin injection (Kaim et al. 1990). Prostaglandin estrus synchronisation is not effective when injection is given during the first five days of the cycle, (ovulation= day 0). Considering this limiatation (Hnasel and Beal 1979) have described two methods for estrus synchronization of heifers. One method involves insemination of all animals coming into estrus for five days after prostagandin treatment with a subsequent injection of prostaglandin to the other animals on day 5; the other method involves two prostaglandin injections 10 to 12 days apart to all heifers. Kaim (1990) reported that 85 % of heifers given two doses of prostglandin 12 days apart came into estrus 5 days following the second prostaglandin injection. First service conception rate in the same study was 61.5 % for synchronized heifers and 82.9 % of the heifers conceived within 30 days of first insemination. Prostaglandin based estrus synchronisation programs are more effective when used with heifers than with cows (Mac Millan et al. 1977, 1978).

Age

Esslemont (1979) considers that there is no general agreement about the relationship between fertility and age, since many authors report decreasing fertility in older cows (Boyd and

Reed, 1961; Van Dieten, 1968 Spalding et. al. 1975, cited by Esslemont, 1979) but some others found no consistent differences in breeding efficiency during the first five lactations (Matsoukas and Fairchild, 1975 cited by Esslemont , 1979)._Decline in breeding efficiency after the fifth lactation was reported for Holstein by Matsoukas and Fairchild (1975) and Morrow et. al. (1966). Under subtropical conditions Gwazdauskas et. al. (1975) reported that fertility, as measured by conception rate, declined with age. In this study conception rates were for virgin heifers, cows in lactations 1 to 4 and cows in lactation 5, 47.6%, 42.7%, 31.9%, respectively. Stevenson et. al. (1983) reported that heifers had an average conception rate for all services of 58 % when compared to cows with 45 % or less. Ray et al. (1992) cited data by Bath et. al.(1978) in which fertility increases to 4 years of age, remains fairly constant to 6 years and gradually declines thereafter. A study conducted by Byereley et al. (1987) showed that the probability of heifers (10.4 to 11.4 months of age) becoming pregnant when inseminated during their first puberal estrus increased with increasing age. However, age did not have any effect on pregnancy rates of heifers bred on their third estrus after puberty.

Body weight and dietary energy

According to Esslemont (1979) there is a general conclusion that bodyweight is less important in affecting reproductive performance of heifers and or cows than a fall or a rise in bodyweight during the breeding season.

Low levels of dietary energy and acute fasting in heifers can inhibit estrus expression and cyclic ovarian function (Bond et. al. 1958; Imakawa et. al. 1983; McCann and Hansel, 1986). Low energy diets induced anestrus or delayed the onset of puberty by suppression of luteinizing hormone (LH) (Imakawa et. al. 1986; Day et al 1986). Imakawa et. al. (1984) reported that a weight loss of

0.111 kg d^{-1} or a slight weight increase of 0.02 kg d^{-1} during 160 days resulted in anestrus of 16 months old beef heifers initially cycling and weighing 290 ± 9 and 298 ± 12 kg, respectively. Knutson and Allrich (1988) working with Holstein heifers at 13.4 months of age and weighing 349±11 kg. did not detect alterations in estrus activity with a moderate feeding restriction, (80 % of NRC energy, protein and DM requirements), that allowed a moderate weight gain of 0.33 kg d^{-1}. Knuston and Allrich (1988) conclude that dietary energy restriction in heifers must be severe and result in weight loss to determine anestrus and cessation of ovarian cyclicity. Butler et. al. (1981) reported that lactating cows losing weight at breeding had decreased fertility, and those gaining weight at breeding showed improved fertility. Foster et. al. (1989) reported that chronic undernourishment of lambs after weaning prevented the development of pulsatile pituitary release of LH, which is expected to cause failure of the onset of estrus or ovulation, however, an increase in pulsatile release of LH occurred after realimentation. An increased concentration of growth hormone (GH) in blood was also observed in the same study, which may be related to pregnancy failure.

Butler and Smith (1989) stated that a negative energy balance in early lactation dairy cows probably acts similarly to undernutrition and may manifest as delayed ovarian activity. Reduced availability of insulin is suggested to decrease luteinizing hormone (LH) pulsatile secretion and to limit ovarian responsiveness to gonadotropins. Release of endogenous opioids in association with increasing feed intake is also proposed to inhibit LH production (Butler and Smith 1989). It has also been postulated that energy deficiency in early lactation itself may be not as important in delaying first ovulation, as the magnitude of the negative energy balance and how fast the cow returns to a positive energy balance status. A small negative balance and a fast recovery to the positive balance would favour ovarian activity. (Butler and Smith, 1989; Butler and Canfield, 1990, Canfield et. al. 1990).

Body conditioning

Esslemont (1979) cited two studies with dairy cows in which a positive relationship between body condition score at service and conception rate was observed. However, excessive body condition at calving has been associated with increased occurrence of metabolic, infectious, digestive and reproductive disorders in early lactation cows (Morrow, 1976; Morrow et. al. 1979). Butler and Smith (1989) observed a larger period to first ovulation and to first service and a reduced first service conception rate in cows with severe body condition loss of more than 1.0 unit (0= thin, 5= obese) during the first 5 weeks of lactation, as compared to cows with minor or moderate loss in body condition (< 1.0 unit). The reduced fertility in cows loosing condition was associated to fat infiltration in the liver, however the mechanism by which fat infiltration affects reproduction is uncertain according to Butler and Smith (1989).

Robertson et. al. (1992) reported that body conditioning modulated the pattern of LH in the circulation, responsiveness of the pituitary to LH releasing hormone (LHRH) and releasable stores of LH in beef heifers weighing 273 ± 3 kg fed diets with low energy. Weight loss in poor body condition (3.9 ± 0.1units, 1-9 scale) heifers resulted increased LH pulse amplitude and pituitary responsiveness to LHRH. Weight loss in high body condition (7.6 ± 0.2 units) heifers, resulted in an reduction of releasable pools of LH but not in alterations of LH releasing pattern and LHRH responsiveness.

Dietary minerals and vitamins

Vitamin A deficiency in dairy cows may cause reduced conception rates, but the major reproductive problems include an increased rate of abortions and the birth of dead, weak or blind calves (Hurley and Doane, 1989). A function independent of Vitamin A has been attributed to β-carotene, the precursor of Vitamin A. A positive effect of β-carotene supplementation on estrus intensity, conception rate as well as a reduction of follicular cysts frequency of dairy catlle is mentioned by Mc Dowell, (1989). However other researchers (Folman et. al. 1979, Wang et. al 1988 cited by McDowell, 1989) found no effect of β-carotene supplementation on reproduction. The carotene requirements suggested by NRC (1989) are 10.6 mg (4,240 IU of vitamin A) per 100 kg of live weight for growing animals, and 19 mg (7,600 IU of vitamin A) per 100 kg of live weight for reproduction as well as lactation.

Because of the similar roles as antioxidants that selenium and vitamin E play in metabolism they are usually studied together. Deficiencies in selenium and vitamin E have been associated with retained placentas, metritis , cystic ovaries, and time of uterine involution in cows with metritis (Eger et. al 1985; Harrison et al 1984; Harrison et al 1986; Segerson et al. 1981). The incidence of retained placenta was completely reverted from 38 % to 0 % in cows with low Se plasma levels (< 0.025 ppm), when Se was supplemented during 60 days prepartum (Julien et. al. 1976). Injection of Se and vitamin E to superovulated cows with inadequate Se status increased the number of fertilized ova as compared to non injected cows (Kappel et al 1984). Some studies, however, working with herds with lower incidence of retained placenta (10%) indicated that supplementation of vitamin E and Se was not effective (Buck et. al 1979, Hidiroglou et al 1987). National Research Council (NRC, 1989) suggests that dairy cattle diets should contain 0.30 ppm of Se and

15 IU of vitamin E per kg of dry diet for mature animals and 15 to 60 IU per kg of dry diet for young calves.

Irregular estrus, lowered conception rate, anestrus, decreased ovarian activity and increased incidence of cystic follicles have been described as consequence of Phosphorus deficiency (Maynard et. al. 1979; Morrow, 1980) Involvement of P in phospholipid and cAMP synthesis is postulated as a key to its effect on reproduction (Hurley and Doane 1989). Phosphorus requirements range from 0.24 to 0.48 % of DM for dairy cows and from 0.23 to 0.31 % of DM for growing heifers and bulls NRC (1989).

Reduced blood calcium at calving and immediately post-partum is associated with an increased incidence of dystocia, retained placenta, and prolapsed uterus, and may delay uterine involution (Morrow, 1980 b, Risco et. al. 1984). The LH release from the pituitary has been reported to involve a Ca-dependent mechanism, which determines that in the absence of Ca or the presence of Ca blocking agents LH is not released (Naor et al. 1981). Excesses of calcium may alter fertility by reducing the absorption of other minerals like P, Mg, Zn, and Cu (King 1971). Calcium requirements range from 0.39 to 0.77 % of DM for dairy cows and from 0.29 to 0.52 % of DM for growing heifers and bulls NRC (1989).

The role of zinc in reproduction may be associated to its contribution as an activator of enzymes involved in steroidgenesis (Robinson, 1989). Zinc supplementation has been shown to increase conception rate in heifers (Piper and Spears 1982). However, zinc requirements on forage-based diets for reproduction are not well defined (NRC, 1996). The national Research Council (NRC, 1989) recommends a level of 30 ppm as an adequate concentration in dairy cattle diets.

Copper has been reported to be involved in maintaining the activity of hypophyseal hormones in blood and to facilitate prostaglandin action by enhancing prostaglandin receptor binding (Georgievskii, 1981 and Barnea et. al. 1985 cited by Hurley and Doane, 1989). Several reproductive disorders are associated with Cu deficiencies including early embryonic death, delayed estrus, reduced conception rates, retained placenta and calving difficulty (Herd 1994, Hidiroglou, 1979). The National Research Council (NRC 1989) recommendation for dairy cattle is 10 ppm in the diet., however if the diet is high in other minerals as S, Fe, Ca, Zn and Mo it may be insufficient because a reduction of copper availability (Hurley and Doane, 1989). National Research Council (1996) recommends 10 ppm of copper in the diet of beef cattle provided S and Mo do not exceed 0.25% and 2mg kg^{-1} diet DM, respectively. Inappropriate Cu:Mo ratios have been reported to delay the onset of puberty, to cause anestrus and increased non-return rates in bovines (Roberts, 1971; Peterson and Waldern, 1977).

Iodine deficiency may affect reproductive performance indirectly by affecting thyroid function. Fetal development can be affected at any stage of gestation, resulting in early embryonic death, fetal resorption, abortion or birth of goitrous calves (Hurley and Doane 1989). The National Research Council (NRC 1989) recommends 0.5 ppm of iodine in the diet.

Manganese is needed to synthesize the mucopolysaccharide in cartilage and bone (Underwood, 1977), to activate enzymes required for carbohydrate and lipid metabolism and may affect fertility directly because its involvement with steroid hormone synthesis (Benedict et. al. 1965 cited by Hurley and Doane, 1989). Manganese deficiency manifestations are rare in ruminants, however, estrous cycle disturbances and low fertilization rate have been reported as Mn deficiency symptoms (Georgievsky et al. 1981). Normal growth in calves can be achieved with

10-15 mg Mn kg^{-1} of dietary DM, however normal reproductive function is suggested to be attained at doses of 30 mg kg^{-1} of DM in the diet or more. (Georgievsky, 1981).

Cobalt is required by the rumen microorganisms for vitamin B12 synthesis (Georgievskii, 1981). Cobalt deficiency is associated with anemia and general unthriftiness which might indirectly result in infertility (Hurley and Doane 1989). The most common effect on reproduction of Co deficiency is reduced conception rate (Hidiroglou, 1979). Recommended Co content of dairy diets dietary is 0.10 ppm on DM basis (NRC 1989).

Season and Temperature

Reproductive performance is negatively affected by high ambient temperatures. Data from a five year study in Arizona by Ray et al (1992) show more services per conception and longer calving intervals for cows calving in spring and summer than in winter and fall. Services per conception were 2.23, 2.32, 1.89, 1.90 for spring, summer, fall and winter respectively. Calving intervals were 385.6, 386.8, 371.8, 369.0 for spring, summer, fall and winter respectively. Daily average maximum temperatures exceeded 40 ° C during June through August, exceeding the upper critical temperatures (24 to 27 °C) that induce heat stress as reported by Fuquay (1981).

Stevenson et. al (1984) in a study done at Kansas State University under moderate temperatures also found seasonal differences in the fertility of prostaglandin treated Holstein heifers. First service conception rates were 81 and 60 % and services per conception 1.2 and 1.6 in winter and spring respectively. Maximum temperatures the day of prostaglandin treatment were 1.8 and 9.6° C in winter and 20.6 and 20.3 °C in spring for two groups of heifers studied respectively. These authors suggest that winter advantages are associated with lowered temperatures and shorter photoperiod in winter as compared to spring. However, another study does not show any effect of photoperiod in luteinizing hormone (LH), follicle stimulating hormone (FSH) and prolactin secretion or estrous cycle length of Holstein heifers (Rzepkowsky, R.A. et. al. 1982).

Dietary protein

The increased intake and digestibility of high protein diets and the consequent increase in milk production, has established a trend to overfeed protein during peak milk production in many north American dairy herds. Protein excesses in those cases are likely to occur simultaneously with breeding time. (Swanson 1989). A negative effect of excess dietary crude protein (CP) on dairy cows fertility has been reported in many, but not all cases (Claypool et. al. 1980; Edwards et. al. 1980; Ferguson and Chalupa 1989, Carroll et al. 1988, Canfield et. al 1990). Reduced fertility was also observed in Holstein heifers fed exceeded ruminally degradable protein. (Elrod et. and Butler, 1993).

Ferguson and Chalupa (1989) summarized the results of several studies that showed increased services per conception and number of days open after calving when dairy cows were fed high protein diets (17-20 % of DM) as compared to moderate protein diets (15-16% of DM). However, increases were not consistent in magnitude, and in some cases an effect on services per conception did not correspond with any effect on days open. In the same publication Ferguson and Chalupa, reviewed the published reports that examined the relationship between crude protein in feed dry matter (CPDM) and fertility in dairy cows. Mathematically modelling the data set of these studies they concluded that there was not a consistent change in conception rate to increasing

CPDM. However, an effect of protein on fertility was not dismissed. Much of the inconsistency observed was solved by considering the fate of consumed protein in the rumen. Conception rates were influenced by degradable and undegradable protein intakes relative to requirement. Increasing degradable and undegradable intake protein relative to requirements decreased the probability of pregnancy. Age and dietary concentration of energy were reported to be modifiers of the impact of protein on reproduction.

Most of the ammonia that arises from the digestion of high protein diets in the rumen is metabolized to urea by the liver. However, maximal capacity to detoxify ammonia can be exceeded with high protein diets leading to elevated blood concentrations of ammonia as well as urea. (Ferguson and Chalupa, 1989). Elrod et. al. (1993) found that plasma urea nitrogen (PUN) of Holstein cows in early lactation was increased by dietary excesses of undegradable intake protein (UIP) and degradable intake protein (DIP) relative to requirements. Elrod and Butler (1993) found a similar effect on PUN when nulliparous Holstein heifers were fed a diet exceeding ruminally degradable protein requirements. Jordan et. al (1983) found increased concentrations of ammonia in blood of Holstein cows fed 23 % CP as compared with 12 %.

There is evidence that increased concentrations of nitrogen metabolites in blood results in increased concentrations of the same metabolites in the reproductive tract. Urea is a small molecule that equilibrates between reproductive tract and plasma (Canfield et. al. 1990). A significant positive relationship between urea in uterine fluids secretions and plasma urea was found when two levels of CP (12%-23%) were fed to high producing Holstein cows (Jordan et. al 1983). These observations and the report by Ferguson et. al. (1988) referring to a plasma urea nitrogen (PUN) level of 20 mg dl $^{-1}$ as a limit above which reduced conception rates can be expected in dairy cows, suggest that nitrogen metabolites may act at reproductive tissue level to impair fertility. Several studies have described the impact of nitrogen metabolism byproducts on fertility of dairy cows, five of which are summarized in table 1.

The data from the studies presented in table 1 show different effects of PUN on fertility. The more consistent effects were described by Ferguson et. al. (1993) in which a significant decrease in CR was observed as a consequence of increasing levels of PUN. A decrease in first service conception rate was also found by Canfield et. al. 1990 when PUN increased from 12.3 to 19.3 mg dl^{-1}. A tendency ($P< .10$) for greater days to first ovulation was reported by Carroll et al. (1988). When PUN increased from 10 mg dl^{-1} to 24.5 mg dl^{-1}. Barton et. al. (1996), showed no effect of increasing PUN on reproduction, except on days to first ovulation that increased only for cows with high PUN and reproductive disorders like ovarian cysts or systemic metritis (21.5 vs. 25.6 d, $P< 0.10$). These results are similar to data reported in the study by Carroll et al (1988) who suggest an interaction between CP intake, lactation number and the occurrence of reproductive health disorders. In one study by Howard et al (1987) where cows were managed with a controlled sanitary program including treatment of uterine infections and ovarian cysts, concentration of protein in the diet did not affect, days open after calving, services per conception and percentage of cows pregnant. Only one study with nulliparous dairy heifers was found in the bibliography reviewed, in which an increase in PUN level (17.5 mg dl^{-1} vs. 23.6 mg dl^{-1}) resulted in a significant decrease in pregnancy rate (82.0 % bred vs. 61.0 % bred) (Elrod and Butler 1993).

Several hypothesis have been elaborated to explain the effects of dietary CP on dairy cows-heifers fertility. Elevated concentrations of ammonia, urea, or other by-products of N metabolism have been suggested to negatively impact the reactivity of lymphocytes. Cows fed high

Table 1. Comparative effects of increasing plasma urea nitrogen on fertility of dairy cows[a].

Study	CP (% DM)	PUN/SUN (mg/dl)	1CR (% bred)	CR (% bred)	PR (% bred)	DFO (Days)
1	16.5- 21.0	8.4 **	-	54.5 **	88.7	-
2	13.0	8.6 ***	40.6	-	75.0	23.2
3	13.0	10.0 ***	64.0	-	96.0	17.0 * _
4	16.0	12.3 **	48.8 **	-	-	27.0
1	16.5-21.0	12.7 **	-	45.6 **	84.6	-
5	14.5	14.5 ***	-	-	86.5	-
1	16.5-21.0	16.8 **	-	42.9 **	85.7	-
4	19.0	19.3 **	31.0 **	-	-	30.9
2	20.0	21.0 ***	43.7	-	87.5	25.8
1	16.5-21.0	21.9 **	-	43.4 **	87.8	-
3	20.0	24.5 ***	56.0	-	-	22.0 *
5	19.4	25.0 ***	-	-	84.8	-
1	16.5-21.0	27.2 **	-	30.4 **	78.0	-

[a] Data is sorted by ascending levels of PUN.

Study 1 = Ferguson et. al. 1993; Study 2 = Barton et. al. 1996; Study 3= Carroll et. al. 1988; Study 4= Canfield et. al. 1990; Study 5=Howard et. al. 1987. CP= crude protein; PUN=plasma urea nitrogen; SUN= serum urea nitrogen; 1CR=first service conception rate; CR=overall conception rate; PR=pregnancy rate; DFO=days to first ovulation after calving; *=P<.10 within each study; **=P<.05 within each study; ***=P<.01 within each study.

CP diets may suffer a suppression of their immune system impairing their health status and reproductive efficiency (Barton et. al. 1996). Targowski et al. (1984) reported that subtoxic concentrations (5 to 10 μg ml^{-1}) of ammonia affected bovine lymphocyte responsiveness.

Toxic local effects of ammonia and urea on sperm, ovum or developing embryo have been mentioned by Canfield et. al. (1990). Bishonga et. al. (1994) concluded that high levels of rumen degradable protein are detrimental to early development and survival of sheep embryos. Decreased ability of sperm to migrate through cervical mucus was found with excessive urea concentrations (100 mg dl^{-1}) (Breau et. al. 1985). Urea has been shown to be toxic to sperm and ova and can cause abortion when injected intra-amniotically (Dasgupta et. al., Umezaki et. al. and Greenhalf and Diggory, cited by Ferguson and Chalupa 1989). Blanchard et al. (1990) reported data obtained from superovulated Holstein cows suggesting that feeding excessive amounts of rumen degradable protein may result in fertilization failure or early degeneration of embryos.

The inhibition of the binding of LH to the corpus luteum receptors as a consequence of the presence of urea nitrogen was mentioned as a possible mechanism to decrease serum progesterone concentration (Jordan et al. 1983). Progesterone is needed to maintain pregnancy and some studies have found that high concentrations of blood progesterone during the estrous cycle previous to insemination were associated to high CR (Folman et. al. 1981; Fonseca et. al 1983). Serum progesterone concentrations were decreased in cows fed moderate and high CP as compared to low CP (16.3% and 19.3 % vs. 12.7 %) (Jordan and Swanson 1979 b). However, Carroll et. al. (1988) reported similar results only in cows with reproductive health disorders.

Alteration of the reproductive tract environment by high circulating N metabolism byproducts was suggested as a possible cause of reduced fertility in some studies. Increased concentration of ammonia in blood and urea in plasma of Holstein cows resulted in increased concentration of urea and decreased concentrations of Mg, P, K, in uterine secretions of a study done by Jordan et. al. (1983). A limitation on the transport mechanism of P and K from blood to the ingravid uterus was speculated, since elevated blood concentration of P and K were found in cows fed a high protein diet. Hurley et al. Cited by Jordan et al. (1983) have reported infertility in rats with severe Mg deficiency. Plasma zinc increased during the first experimental estrous cycle in cows with high ammonia and PUN. Elrod and Butler (1993) observed that uterine pH of Holstein heifers fed high protein diets was significantly lower than heifers fed low protein diets during the luteal phase. These researchers suggest that the differences in pH observed in their study reflect alterations in the uterine secretory activity but could not determine the mechanisms involved.

Excesses of amino acids relative to requirement may have an effect on reproduction by indirectly affecting energy balance and nutrient partitioning (Ferguson and Chalupa, 1989). Amino acids in excess are deaminated and the amino group converted to urea which requires energy. Diets with excess undegradable protein and marginal energy-yielding nutrients may then exacerbate negative energy balance in lactating dairy cows and delay first ovulation, first breeding and reduce CR. When energy-yielding nutrients are adequate a greater impact on fertility may be expected on young cows rather than older cows, considering the drive to achieve mature size which partitions absorbed nutrients to growth (Ferguson and Chalupa, 1989). Bruckental et. al. (1989) observed higher pregnancy rates in cows fed a diet containing a protein source (fish meal) with low rume degradability as compared to cows fed a diet with similar CP but including a highly degradable protein source (soybean meal).

HEIFER REARING IN URUGUAY

The high dependence on pasture feeding for Uruguayan dairy herds, and the seasonal variations in quality and productivity of pastures suggest that shortages and/or imbalances in the diets are likely to occur. Under traditional rearing conditions where native pasture without supplementation is the rule, low energy and protein availability during winter cause growth retardation. Puberty is usually reached at older ages than reported in the literature, and heifers are bred for the first time at about two years of age. Fertility problems would be expected to occur during winter breeding when poor body conditions and potential body weight loss can occur. Moderate weight gains should occur during the spring months which are expected to contribute to better reproductive performance. Phosphorus deficiencies are likely to occur on spring-summer native pastures (Berreta 1996) and may cause reduced fertility if no P mineral supplementation is supplied. Availability of other minerals is not well quantified in Uruguayan native pastures, and may also affect reproductive performance. Shortages of Vitamins A and E may occur during winter when cattle are pastured on dormant summer species.

Intensive heifer rearing systems have been implemented in some commercial dairy farms, and more recently on specialized heifer rearing farms. In these cases, legume pastures, mineral supplementation, and occasional concentrate, hay or silage supplementation contribute to improve the quantity and quality of the diets. Better weight gains are expected in these situations, however, puberty is still retarded on heifer farms since heifers usually come from average commercial herds where the initial growth (birth to 6 - 8 months) is not adequate. Fertility restrictions in these systems may arise from nutrient imbalances or deficiencies in pastures. During the spring breeding season heifers are fed exclusively on legume pasture which normally contains more than 17 % crude protein. During the winter pastures may be supplemented with corn silage or pasture hay. Mineral shortages of great importance are not expected to occur since mineral salts are usually offered ad libitum.

Ambient temperatures are usually not as severe as in the report by Ray et. al (1992), nevertheless, during spring-summer services maximum temperatures may affect fertility. The mean temperatures of the warmest month (January) range from 22 to 27 ° C (south to north), and of the coldest month (July) from 11 to 14 ° C (south to north), while the mean maximum temperatures of January range from 27.7 to 32.6 (south to north) and of July from 15.5 to 18.9 (south to north) (Corsi, 1978).

Considering the effects of age on first breeding observed by Byereley et. al. (1987), if adequate feeding is done during breeding to allow adequate weight gains, growth retarded heifers have a good chance of becoming pregnant, even during their first pubertal estrus.

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Breeding performance of dairy cows and heifers under current management practices in Uruguay

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Abstract

An analysis of breeding performance was conducted for Uruguayan dairy cattle systems based on pasture feeding. Management and reproductive data of 602 artificially inseminated dairy cows were obtained from the 1994 and 1995 records of two dairy farms (farms 1 and 2), and of 3696 artificially inseminated heifers from the 1993 and 1994 records of two heifer rearing farms (farms 3 and 4). Data from 1994, utilising legume pasture had an overall pregnancy rate (% of bred) of 82.2% and 88.0%, first service conception rate of 43.2 and 50.3%, 2.20 and 2.25 services per pregnancy, for farms 1 and 2, respectively. Under a supplemented pasture feeding regime (1/3 legume pasture, 1/3 corn silage and 1/3 grain, DM basis) during 1995, the overall pregnancy rate (% of bred) was 90.1%, first service conception was 62.7% with 1.63 services per pregnancy on farm 1. The improved breeding performance observed from 1994 to 1995 was coincident with a reduction in herd blood urea nitrogen (BUN), 25.9 and 16.5 mg dL^{-1} for 1994 and 1995, respectively. Farm 3, using legume pasture, had better breeding performance in heifers during the winter as compared to the spring service period. Overall conception rates were 88.9, 82.8, 96.1 and 82.2%, first service conception was 75.1, 70.7, 78.3 and 71.5%, with 1.33, 1.40, 1.27 and 1.42 services per pregnancy for winter 1993, spring 1993, winter 1994 and spring 1994, respectively. Early pregnancy diagnosis, done by ultrasound on farm 3 in 1994 for a restricted number of heifers showed that 1.9 and 4.4% of the embryos were lost for the winter and spring service periods, respectively. Farm 4, also using legume pasture had better breeding performance in the spring as compared to the winter service period. The overall conception rate was 78.6, 82.8, 84.1 and 89.1%, first service conception was 63.5, 74.4, 72.8 and 72.3%, with 1.81, 1.45, 1.46 and 1.43 services per pregnancy for winter 1993, spring 1993, winter 1994 and spring 1994, respectively. Herd analysis data suggest that seasonal variation for breeding performance does exist and that those differences may be related to the diet. Further work is required to establish critical factors affecting reproduction of cattle under Uruguayan management.

Key words: Uruguay, dairy, breeding performance, legume pasture, cows, heifers.

Abbreviation key: **AI**= artificial insemination, **BUN**= blood urea nitrogen, **CARECO**= Campo de Recria Colonia, **CONAPROLE**= Cooperativa Nacional de Productores de Leche, **CP**= crude protein, **CR**= conception rate, **DM**= dry matter, **INIA**= Insituto Nacional de Investigacion Agropecuaria, **PUN**= plasma urea nitrogen,

Introduction

Uruguay is located in South America between latitudes 35° and 30° South. Average precipitation is 930 mm yr^{-1} in the south and 1300 mm yr^{-1} in the north. Average precipitation data over more than 40 years indicate that rainfall is equally distributed during the year (29, 24, 23 and 23% in the south, and 29, 17, 27, 35% in the north during the fall, winter, spring and summer seasons, respectively), however, there is great variability among years (Corsi, 1978). The mean temperature of the warmest month (January) ranges from 22 to 27 °C (south to north), while the mean temperature of coldest month (July) ranges from 11-14 °C (south to north, Corsi, 1978). The mild, temperate climate supports a dairy farming system based on year round pasture grazing.

Improvements achieved in milk yield in the last two decades have been directly related to an increased use of improved pastures, mainly exotic legume species such as white and red clover, and birdsfoot trefoil. The higher dry matter (DM) yield and better quality of these pastures has improved the nutritional status of dairy cattle relative to growth, lactation and overall breeding performance. However, seasonality of pasture production and year to year variation in climate has caused a diversity of animal production responses and resulting feeding strategies.

The former practise of breeding dairy cows on a year round basis is changing. Most farms currently manipulate their breeding schedule in order to adapt to market needs and pasture forage production. Today, breeding usually takes place from May to January with one peak in the fall-winter months of May-July and another in the spring-summer months of November-January. Usually there is no breeding from February to April to avoid summer calving when pasture quality is poor for peak lactation cows. Thus there is a big concentration of calvings in the fall and a smaller one in spring. Peak milk production is achieved in the spring months, September to December, when pastures approach the maximum rate of DM production. Legume pasture is frequently the sole component of diets for lactating cows in the spring. The lactation ration during the fall and winter months is legume pasture and winter forage crops grazed in early vegetative stage such as oats, ryegrass, and forage wheat which provide most of the protein requirements. The winter season has the least pasture forage DM production, requiring farms with high stocking rates to supplement their dairy herds with corn silage and concentrates, mainly to meet the animal's energy requirements. Farm management with in low stocking rates may require less energy and protein supplementation during the winter months, and often harvest excess spring pasture for forage conservation in the form of hay. Plant maturation and decreased quality are expected to occur in December for improved pastures. Low soil water availability, and increased solar radiation during the summer months results in a very low forage biomass yield for legume pastures. Summer forage crops like sudangrass, sorghum and corn are grazed during the summer and provide adequate amounts of DM, but are of a relatively poor quality in terms of protein. High producing herds utilise excess spring pasture conserved as silage together with a limited amount of concentrates to supplement the summer forage crops.

Replacement heifer management is diverse on dairy farms in Uruguay. The general rule is that the best pastures are reserved for lactating cows, and heifers are confined to areas with poor quality improved pastures or native pastures. Energy and protein supplementation for grazing heifers is not common. Poor rates of growth are obtained under such conditions.

First service is done when heifers reach 300 - 320 kg, which occurs at 24 - 30 months of age. Many farmers do not keep their heifers on the farm after weaning. As a result, specialised farms for heifer rearing have developed over the last 10 years, where better replacement heifer management is pursued.

Reproduction efficiency in Uruguay's dairy herds is not well documented but assumed to be acceptable. However, difficulties to impregnate cows and heifers have been observed in certain situations. As a consequence, service periods must be extended or some animals have to skip a breeding season, which implies a long period of low or no production.

Cavestany (1993) describes the pregnancy rates of 1990 and 1991 of the experimental herd at La Estanzuela Experiment Station of the Instituto Nacional de Investigacion Agropecuaria (INIA). Overall pregnancy rate as a percentage of total cows was 71.1% in 1990 and 58.2% in 1991. The first service conception rate was 45.1% and 50.6% for 1990 and 1991, respectively. A 30 day service period was used during the fall for experimental purposes, while a 90 day service period was used for spring and winter breeding in both years. In 1990, the overall pregnancy rate of 65 cows in the fall, 88 in winter and 73 in spring were 56.9, 82.9 and 69.8% respectively. In 1991, the overall pregnancy rate of 67 cows in the fall, 80 in winter and 75 in spring were 73.1, 62.5 and 40.0% respectively (Cavestany 1992). Breeding performance of dairy heifers at the same Experiment Station also was described by Cavestany (1992). A pool of breding data from 1991 and 1992 indicate pregnancy rates as percentage of total heifers to be 68% for 25 heifers in winter and 40% for 35 heifers in spring.

Qualitative aspects of local feeding strategies are pointed out as a possible restriction to breeding performance. A high crude protein (CP) content in improved pastures, and the dependence on pasture as the sole component of the diet in spring have been implicated as one reason for poor breeding performance during this season (Cavestany 1992).

With the exception of records provided by INIA, little data has been published on the reproduction efficiencies of Uruguayan dairy herds. Therefore, a herd analysis was conducted to collect breeding data from two commercial dairy herds and two heifer rearing farms. The first objective of this survey is to obtain and evaluate data relative to dairy cattle reproduction in Uruguay. A second objective of the survey is to establish the degree of year to year variation and season to season variation that may be experienced with current production practices.

Materials and Methods

Breeding and management data from two dairy farms (Farm 1 and Farm 2) and two heifer rearing farms (Farm 3 and farm 4) were collected. The veterinary services of the National Dairy Farmers Co-operative (CONAPROLE) provided the data for Farms 1 and 2. The herd veterinarian responsible for breeding provided farm 3 data. Farm 4 data was provided by the Colonia Heifer Rearing Co-operative (CA.RE.CO). These four farms are located within the main dairy producing area in southern Uruguay. Data collected correspond to 1994 and 1995 for farm 1, 1994 for farm 2, and 1993 and 1994 for farms 3 and 4.

Farm 1 managed 320 Holstein cows in 1994 and 293 in 1995 within a pasture land area of 414 ha. Most of the breeding was done by artificial insemination (AI), without estrus synchronization and standing heat was detected visually. Clean up bulls were used on cows repeating heat for the fourth time. Artificial insemination was done approximately 12 hours after heat detection. Farm 2 managed an average of 130 Holstein cows in 1994 within an area of 450 hectares. Cow breeding management was similar to farm 1 using AI without estrus synchronization and visual detection of standing heat.

In farm 3, an average of 1000 Holstein heifers were reared on a 1000 hectare farm. Heifer breeding was done by AI, estrus synchronisation with prostaglandin was done on a routine basis, and standing heat was detected visually. An average of 1800 Holstein heifers were reared on 2500 hectares for farm 4, also with AI, estrus synchronization and visual detection of standing heat.

The management information collected included feeding programs for all farms; yearly milk production, and average number of cows in lactation per year for farm 1; and heifer weight and reproductive status prior to breeding for farms 3 and 4.

Breeding information included heat detection, AI strategies and pregnancy diagnosis methods utilised on all farms. Breeding records from all farms were also reviewed. Data collected for farms 1 and 2 included start and end dates of the breeding seasons, number of cows inseminated per bull, bull identity and number of cows pregnant at first service. Data collected for farms 3 and 4 include insemination date, bulls used in each insemination, name of inseminator, prostaglandin treatment date, pregnancy diagnosis and dates of pregnancy diagnosis. Blood urea nitrogen data from farm 1 obtained during the first 100 days of lactation in 1994 and 1995 were also provided by CONAPROLE.

Breeding season length, first service conception rates, overall conception rates, and number of services per pregnancy were calculated from the breeding records of farms 1 and 2. Milk production per cow on farm 1 was calculated by dividing the total amount of milk produced in the year by the average number of cows in lactation during the whole year. Breeding season length, percentage of cycling heifers, service conception rates, overall conception rates, overall pregnancy rates and number of services per pregnancy were calculated for farms 3 and 4. Cycling heifers are defined, for the purposes of this manuscript, as the proportion of heifers observed in heat relative to the number of heifers brought in for breeding.

Conception rate is defined as the percentage of heifers or cows not repeating heat after a specific service and diagnosed pregnant by rectal palpation, relative to the total number of animals inseminated in that specific service. Overall conception rate is defined as the percentage of animals diagnosed pregnant relative to the total number of animals inseminated. Overall pregnancy rate is defined as the percentage of all animals diagnosed pregnant relative to the total number of animals in the breeding herd. Number of services per pregnancy was calculated dividing the total number of services by the number of animals diagnosed pregnant. Percentage of abortions was calculated for farm 4 by subtracting the number of heifers diagnosed pregnant at first palpation from the number of heifers diagnosed pregnant at second palpation, and expressing it as a percentage of heifers diagnosed pregnant at first palpation. Services per bull as percentage of total services were calculated for all the breeding seasons evaluated in all the farms.

Seasonal and year effects on the breeding performance for heifer farms were analysed by comparison of proportions using contingency tables and chi square analysis (Steel and Torrie 1980).

Results

Legume pasture composed of red clover *(Trifolium Pratense)*, white clover (*Trifolium repens)*, and birdsfoot trefoil (*Lotus corniculatus)* was fed to Farm 1 and 2 dairy herds during the 1994 breeding season. This feeding strategy was changed in 1995 for farm 1 to avoid a sole pasture diet by feeding a 1/3 legume pasture (same species), 1/3 corn silage and 1/3 grain mixture (corn, wheat, barley), DM basis. Salt supplementation (NaCl) was provided on an ad libitum basis on farm 1 in both years. No accurate information was obtained from farm 2 relative to the mineral supplementation. Heifers from farms 3 and 4 were fed legume pasture composed of white clover and red clover during the breeding seasons evaluated. No mineral supplementation was offered on farms 3 and 4.

Milk production on farm 1 was 1,134,268 l and 1,113,382 l yr^{-1} in 1994 and 1995, respectively. The number of lactating cows averaged 183 and 170 in 1994 and 1995, respectively. The calculated average milk production per cow was 6198 and 6549 l in 1994 and 1995, respectively. No accurate information was available for milk production and average number of cows in lactation in farm 2.

The 1994 breeding season started on June 4 and ended December 30 (207d); and in 1995, the breeding season started on June 6 and ended December 28 on farm 1. In 1994 and 1995, respectively 264 cows and 233 cows were inseminated during the whole season. On farm 2 the breeding schedule was from May 20 to September 10 (113 d). One hundred and five cows were inseminated during the whole season.

Winter breeding on farm 3 occurred from June 5 to July 8 (33 d) in 1993, and from June 8 to July 20 (42 d) in 1994, while spring breeding occurred from November 8 to

December 11 (33 d) in 1993 and from November 7 to December 23 (46 d) in 1994. In total 258 heifers were brought for breeding in winter 1993, 262 heifers in winter 1994; 234 in spring 1993 and 432 heifers in spring 1994.

Winter breeding on farm 4 was done from May 25 to August 12 (80 d) in 1993 and from May 23 to August 16 in 1994, while spring breeding took place from November 19 to January 20 (72 d) in 1993 and from November 21 to January 27 (67 d) in 1994. In total 732 heifers were brought for breeding during winter 1993, 901 during winter 1994; 425 heifers in spring 1993; and 473 in spring 1994.

Estrus was visually detected on all surveyed farms. Cows or heifers demonstrating a standing heat after being mounted by their herdmates were considered in heat. Estrus was observed twice a day on farms 1 and 2. Heat detection on farm 1 was done at sunrise (approximately 7:00 h in winter and 5:30 h in spring) and 1 hour before sunset (approximately 17:00 h in winter and 18:30 h in spring), for 1 hour each time. Heat detection on farm 2 was done when cows were taken from pasture to the milking parlour and during milking, 8:00 to 11:00 h in the morning and 20:00 to 23:00 h in the evening. On both farms, cows observed in heat in the morning were inseminated before the evening milking, and cows observed in the evening were inseminated before morning milking of the following day. Heifers were rounded up twice a day, at sunrise and 1 hour before sunset, and observed for 1 hour for heat detection on farms 3 and 4. Heifers in heat in the afternoon were inseminated after morning heat detection, while heifers in heat in the morning were inseminated before afternoon heat detection.

Breeding was done by one technician per breeding season on farms 1, 2 and 3 and by 2 technicians on farm 4. On farms 1 and 2 observed heats were spontaneous, since there was no estrus synchronisation. Cows were inseminated until the end of the breeding season as many times as they were observed in heat on farms 1 and 2. No data was available on the number of cows not showing signs of estrus at these locations.

Twenty-one bulls were used on farm 1 in 1994 and 19 in 1995. On farm 2, 8 bulls were used on the evaluated breeding season in 1994. Services per bull as a percentage of all inseminations are described in Appendix table 1.

Heifers were weighed at the beginning of each breeding season on farm 3. In both years heifers, weighing more than 300 kg in winter and more than 320 kg in spring were included in the insemination group. Once the service period started, spontaneous estrus was detected for five days (day 0= first day of estrus detection), on day 6 the heifers that were not inseminated were examined by rectal palpation to eliminate from the group all infertile, pregnant and sexually underdeveloped heifers. Heifers staying in the group and not inseminated during the first 5 days were synchronised using a single injection of prostaglandin. Estrus detection and insemination continued until day 10 in 1993. No heat detection or insemination were done between day 11 and day 16. A rectal palpation was done on day 17 on non-inseminated heifers. Non-cycling heifers, as determined by the absence of a functional corpus luteum by rectal palpation, were eliminated from the group, while cycling heifers were injected with prostaglandin again. A second insemination round of 18 days, with injected heifers and repeating heifers from the first round, was initiated on day 17. The heifer selection process in 1994 was similar to that of 1993. The first insemination round was similar to 1993 but lasted 13 days instead of 10. No heat detection and insemination were done between day

14 and day 39. Ultrasound pregnancy diagnosis was done for all inseminated heifers on day 39. A prostaglandin injection was given to synchronise heifers diagnosed open and heifers not inseminated during the first round on day 39, starting a new insemination round of 6 days in winter and 8 days in spring.

Semen from the same 4 bulls were used in both winter seasons: David, Emperador (Uruguayan bulls), 5720 and 6037. During spring breeding four bulls were used in 1993: 2531, 924 David and Galstar; and 4 bulls in 1994: David, 2134, 1960 and 546. Bull semen was randomly assigned to the heifers (Appendix 2).

Heifer selection for insemination on farm 4 was done according to weight in the same way as on farm 3. However, the insemination strategy was different. Rectal palpation was done before starting the breeding periods. Only cycling heifers were kept in the group. Estrus detection and insemination started the day heifers were injected and ended 6 days after for the first insemination round during the four seasons evaluated. Two further rounds of estrus detection and insemination were done at the time open heifers were able to ovulate again. The second round took place from day 15 to day 31, and the third round from day 31 to day 63 in spring 1993 and to day 68 in spring 1994. Due to the large number of heifers involved, two groups of animals were managed in the same way but started on different dates for both winter breeding periods. All heifers were included in the second round of heat observation. Heifers already inseminated during the first round and not repeating estrus were separated from the group and held for rectal palpation diagnosis. All heifers showing estrus during the second round of heat observation were inseminated. Heifers inseminated during round 2 and heifers still not served during rounds 1 and 2 were included in round 3. Heifers showing estrus signs during round 3 were inseminated. The service period ended when round 3 was over.

Three bulls were used during the 1993 winter breeding: David, Bovaliant and Emperador; two bulls were used during 1993 spring breeding: David and Bovaliant. In 1994, three bulls were used in winter: Dylan, Willow and Windjammer; and four bulls in spring: David, Willow, Windjammer and Mountain Boy. All bulls were randomly assigned to heifers and services (Appendix 2).

At the end of each breeding season pregnancies were diagnosed by rectal palpation on all farms. On farms 1, 2 and 3, diagnosis was done between 2.0 to 2.5 months after the last insemination in all the seasons evaluated. On farm 4, the first diagnosis was done approximately 48 days after the end of the service period. A second palpation was done 90 to 150 days after the end of the service period to all heifers diagnosed pregnant at the first palpation. The same diagnosis strategy was followed in both years.

Breeding performance results from farm 1 and 2 are described in Table 2. Farm 1 data from 1994 indicates that first service conception rate was 43.2%, with 2.2 services per pregnancy to achieve an 82.2% overall conception rate. Almost 18% of the cows remained open which means that they were maintained until the following year to get pregnant and start a new productive cycle. In 1995, under a supplemented pasture regime, the same herd first service conception rate was 62.7% with 1.63 services per pregnancy to achieve a 90.1% overall conception rate. Blood urea nitrogen (BUN) levels in the cows of farm 1 in 1994 were 25.9 mg/dL. The supplemented diet used in 1995 resulted in a BUN level of 16.5 mg/dL.

Only 1994 data was available for Farm 2. The first service conception rate was 50.5%, with 2.23 services per conception required to successfully impregnate 83.8% of the cows.

Table 2. Breeding performance and blood urea nitrogen of two commercial Uruguayan dairy herds, Farms 1 and 2, in 1994 and 1995.[z]

	1994		1995
	Farm 1	Farm 2	Farm 1
Cow productivity, l yr^{-1}	6198	na[y]	6549
Breeding season, days	211	114	207
Herd size, # of cows	320	130	293
# cows inseminated	264	105	233
First service conception rate[x]	43.2	50.5	62.7
Overall conception rate[x]	82.2	83.8	90.1
Services per pregnancy[w]	2.20	2.23	1.63
Blood urea nitrogen, mg/dl[v]	2585 ± .24	-	16.45 ± .023

[z] Data was provided courtesy of CONAPROLE.

[y] na - data not available

[x] Conception rate = (# cows pregnant / # cows inseminated)*100

[w] Services per pregnancy = total number of services/# cows pregnant

[v] Average values from the first 100 days of lactation

For farm 3, overall conception rates were 88.9% in winter and 82.8% in spring for 1993 (Table 3). In winter 1994, overall conception rate (96.1%), seemed to be better than that observed in winter 1993, however in spring 1994 the observed value (82.2%) was close to that observed in spring 1993 (Table 3). First service conception rates, as determined by rectal palpation, 75 days after breeding, were 75.1% in winter and 70.7% in spring, for 1993 (Table 4). A similar trend for 1st service conception rates in winter relative to spring was observed in 1994 (78.3% and 71.5% for winter and spring, respectively, Table 4). The early ultrasound diagnosis, done 35 days after breeding to the 216 heifers inseminated during the first insemination round, determined that 76.9% heifers were pregnant in winter and 74.4% in spring. However, the proportion of pregnant was reduced to 75.0% and 70.0% for winter and spring respectively when rectal palpation was done 75 days after breeding (Table 5). A low number of services per pregnancy was achieved in both winter periods (1.33 and 1.27) relative to the spring breeding periods (1.40 and 1.42) (Table 3).

Overall conception rates in 1993 and 1994 were similar on farm 4 close to each other. Values observed were 86.4% in winter and 87.3% in spring for 1993, and 87.4% in winter and 90.4% in spring for 1994 (Table 3). First service conception rates, as determined by first rectal palpation 48 days after breeding, were 63.5 % in winter and 74.4% in spring for 1993. The 1994 data suggests a better 1st service conception rate than in 1993 in winter (72.8%), but not in spring (72.3 %, Table 4). The number of services was 1.65 vs. 1.38 in the winter and 1.40 and 1.41 in the spring of 1993 and 1994, respectively (Table 3). The second palpation done 90 to 150 days after breeding indicated variation in the percentage of pregnancy losses after first palpation (abortions), losses ranging from 1.5 to 9.0 % of the pregnancies.

Table 3. Breeding performance of two Uruguayan heifer rearing farms in 1993 and 1994. [z]

	1993				1994			
	WINTER		SPRING		WINTER		SPRING	
	CARECO (FARM 4)	CONAPROLE (FARM 3)	CARECO (FARM 4)	CONAPROLE (FARM 3)	CARECO (FARM 4)	CONAPROLE (FARM 3)	CARECO (FARM 4)	CONAPROLE (FARM 3)
# Heifers brought into	732	258	425	234	901	262	473	432
# Farms where heifers originate	94	na[y]	90	na	102	na	85	na
Body weight, kg	Na	na	282.8 ± 34.2	na	322.4 ± 41.4	400.0 ± 32.6	337.8 ± 37.6	na
Breeding season, days	80	34	63	35	86	45	68	47
# Heifers inseminated	616	217	402	232	868	231	452	404
Overall conception rate [x]	86.4	88.9	87.3	82.8	87.4	96.1	90.4	82.2
Overall pregnancy rate [w]	72.6	74.8	82.6	82.1	84.2	84.7	86.5	76.9
Abortions[v]	9.0	na	5.1	na	3.8	na	1.5	na
Services /pregnancy[u]	1.65	1.33	1.38	1.40	1.40	1.27	1.41	1.42

[z] Data was provided courtesy CONAPROLE and CARECO, data was not statistically analysed

[y] na - data not available

[x] Conception rate= (# heifers pregnant 1st palpation/# heifers inseminated)*100

[w] Pregnancy rate = (# heifers pregnant 1st palpation/# heifers brought into)*100

[v] Abortions = ((# heifers pregnant 1st palpation – # heifers pregnant 2nd palpation)/ (# heifers pregnant 1st palpation))*100

[u] Services per pregnancy = total number of services/# heifers pregnant 1st palpation

Table 4. Heifer breeding performance at 1st, 2nd and 3rd services on two Uruguayan heifer rearing farms in 1993 and 1994[z]

	1993				1994			
	WINTER		SPRING		WINTER		SPRING	
	CARECO (FARM 4)	CONAPROLE (FARM 3)	CARECO (FARM 4)	CONAPROLE (FARM 3)	CARECO (FARM 4)	CONAPROLE (FARM 3)	CARECO (FARM 4)	CONAPROLE (FARM 3)
1st service								
# Heifers inseminated	616	217	402	232	868	231	452	404
# Heifers not returning to heat	431	178	330	195	694	na	345	na
# Heifers pregnant 1st palpation	391	163	299	164	632	181	327	289
Conception rate[y]	63.5	75.1	74.4	70.7	72.8	78.3	72.3	71.5
2nd service								
# Heifers inseminated	185	39	72	37	174	52	107	68
# Heifers not returning to heat	111	na	64	na	154	na	87	na
# Heifers pregnant 1st palpation	108	30	46	28	114	41	71	43
Conception rate	58.3	76.9	63.8	75.7	65.5	78.8	66.3	63.2
3rd service								
# Heifers inseminated	74	na	8	na	20	na	20	na
# Heifers pregnant 1st palpation	33	na	6	na	13	na	11	na
Conception rate	45	na	75	na	65	na	45	na

[z] **Data was provided courtesy of CONAPROLE and CARECO.**

[y] **na - data not available.**

[y]**Conception rate= (# heifers pregnant 1st palpation/ # heifers inseminated)* 100**

Table 5. First service breeding performance of a group of heifers on one Uruguayan heifer rearing farm with ultrasound pregnancy diagnosis in 1994[z]

	Winter	Spring
# Heifers inseminated	216	277
# Heifers pregnant (ultrasound)	166	206
# Heifers pregnant (palpation)	162	194
Conception rate (ultrasound)[x]	76.9	74.4
Conception rate (palpation)	75.0	70.0

[z] Data was provided courtesy of CONAPROLE.

[x] Conception rate = # heifers pregnant (ultrasound or palpation)/ # heifers inseminated

The breeding response was variable between the two seasons and the two years evaluated (Tables 6 and 7). A better response was observed in spring relative to winter (P< 0.01) and in 1994 relative to 1993 (P< 0.01) in terms of overall pregnancy rates and proportion of heifers observed in heat (cycling). Season and year did not affect overall conception rate (Tables 6 and 7).

Discussion

The seasons selected for breeding cows and heifers (winter and spring) were consistent with the Uruguayan recommendations to obtain the best lactation performance. A study conducted by Faggi et al. (1978) comparing lactation curves of cows calving through out the year has shown that the largest milk yields are obtained with cows calving in the fall and winter as compared to the spring and summer; cows calving in May and June producing the largest amounts of fat-corrected milk.

Schmidt and Van Vleck (1975) established that first service conception rates in healthy cows should be 70%. However, data from other studies reveals that actual values are usually lower. Reproductive data from 91 British herds surveyed during one year by Esslemont (1992) reveal an average of 50.5%, while Schmidt and Van Vleck (1975) describe data from five studies with cows served 60 to 90 days post-calving that averaged 61.6% for first service conception rates. Morrow (1980a) establishes that reproductive programs should achieve at least a goal of 45-55% first service conception rate.

Wattiaux (1996) considers that 1.8 or less services per pregnancy is desirable, which indicates good bull and cow fertility and adequate insemination management, while more than 2.5

Table 6. Seasonal breeding performance of heifers from two Uruguayan rearing heifer farms[z].

	Winter	Spring	P-value[y]
# of heifers	2153	1564	-
# of heifers inseminated	1932	1490	-
# of heifers pregnant	1706	1284	-
Cycling heifers, %	89.7	95.3	<0.01
Overall conception rate[x]	88.3	86.2	0.06
Overall pregnancy rate[w]	79.2	83.0	0.03
Services per pregnancy[v]	1.45	1.41	-

[z] Data was provided courtesy of CONAPROLE and CARECO.

[y] P values are determined using chi-square analysis between treatments, df=1.

[x] Overall conception rate= (pregnant heifers at 1st palpation/# heifers inseminated)*100

[w] Overall pregnancy rate = (pregnant heifers at 1st palpation/# heifers brought into)*100

[v] Services per pregnancy = total number of services/# of heifers pregnant at 1st palpation

Table 7. Yearly breeding performance of heifers from two Uruguayan heifer rearing farms.[z]

	1993	1994	P- value[y]
# of heifers brought in	1649	2068	-
# of heifers inseminated	1467	1955	-
# of heifers pregnant	1268	1722	-
Cycling heifers	89.0	94.5	<0.01
Overall conception rate[x]	86.4	88.1	0.15
Overall pregnancy rate[w]	76.8	83.3	<0.01
Services per pregnancy[v]	1.49	1.39	-

[z] Data was provided courtesy of CONAPROLE and CARECO.

[Y] P values are determined using chi-square analysis between treatments, df=1.

[x] Overall conception rate= (pregnant heifers at 1st palpation/# heifers inseminated)*100

[w] Overall pregnancy rate = (pregnant heifers at 1st palpation/# heifers brought into)*100

[v] Services per pregnancy = total number of services/# of heifers pregnant at 1st palpation

services per pregnancy is evidence of serious reproductive problems. The survey done by Esslemont (1992) determined 1.98 services per pregnancy for the 91 farms studied and 1.9 for the top 22 selected on the basis of calving rate.

A target overall conception rate, is suggested to be 88% by Morrow (1980 a). Esslemont (1992) reported that in his study the overall conception rate was 89.5%, and the overall pregnancy rate 79.8%. Top farms surveyed by the same researcher achieved 92.1% overall conception rate and 85.3% overall pregnancy rate.

Breeding efficiency on Farm 1 in 1994 was poorer than the values reported by the literature. However, under the different feeding strategy used in 1995, overall conception rate and services per pregnancy were better on farm 1 than the average values reported by Esslemont (1992), and target values suggested by Morrow (1980a) and Wattiaux (1996). First service conception rate was similar to the studies cited by Schmidt and Van Vleck (1975) and better than the target established by Morrow (1980a).

Many factors other than nutrition can influence the breeding performance of dairy cows, however, an adverse diet effect through increased circulating urea levels on breeding performance has been speculated in this case. Ferguson et al. (1988) refers to a BUN level of 20 mg/dL as a limit above which reduced conception rates can be expected in dairy cows. Butler et al. (1996) found that the conception rate for dairy cows with BUN levels below 19 mg/dL on the day of insemination was 52.7%, while conception rates for cows with BUN levels above 19 mg/dl was 33.4%. Canfield et al. (1990) reported lower conception rates (31% vs. 48%) and higher BUN levels (19.3 mg/dl vs. 12.3 mg/dL) in cows fed a high protein (19.2%) diet as compared with cows on a low protein diet (16.5%). Better breeding performance was found for farm 1 than in the reported cases, however, conception rates follow a similar trend when comparing 1993 and 1994 BUN levels and breeding performance. First service conception rate was similar to the average values observed by Esslemont (1992). However, services per pregnancy and overall conception rate seem to be poor with respect to the potential goals reported (Wattiaux 1996, Esslemont 1992,).

Pregnancy rates and first service conceptions on both dairy farms were similar to the literature targets, however, more services per pregnancy were observed than what is recommended. Management for farm 1 in 1995 resulted in very acceptable breeding performance, and was above the expectations considering the studies reviewed. The breeding performance observed on farm 1, however, could be considered less acceptable when the milk production levels are examined. Butler and Smith (1989) showed a negative association between milk yield and conception rate in lactating cows. A 35-year survey of New York dairy herds showed that in 1950 average milk production was 4500 kg per cow per lactation, and first service conception rate 66%. In 1985 more than 6800 kg were produced per cow and conception rates declined to 51%. Esslemont (1979) suggests that 7000 l per cow per lactation is a realistic level of production above which fertility is likely to be adversely affected in European herds. From the total milk produced per year in farm 1 an estimation of 5200 to 5400 l per lactation can be calculated. It seems that certain management practices in Uruguayan dairy herds could lead to poorer conception than expected, and that an increased number of services are needed to achieve acceptable pregnancy rates.

Better fertility efficiency in first mating heifers than in dairy cows is generally reported in the literature. The Butler and Smith (1989) survey showed that the 66% first service conception rate of virgin heifers did not change during 35 years (1950- 1985). Stevenson et al. (1983) observed that heifers had an average conception rate of all services of 58% when compared to cows with 45% or less. In an experiment carried out to evaluate embryonic death in nulliparous Holstein heifers, Kastelic et al. (1991) observed a 77.8% first service conception rate 25 days after breeding. Elrod and Butler (1993) observed that first service conception rate, as determined by rectal palpation 45 days after breeding, was 82% for Holstein heifers fed normal levels of protein. Dolezel et al. (1985) using crossbred dairy heifers reported services per pregnancy ranging from 1.65 to 1.46 when first inseminated at body weights ranging from 325 to 369 kg respectively.

Lower first service conception rates than the reported values by Kastelic et al. (1991) and Elrod and Butler (1993) were determined by rectal palpation 48 to 75 days after breeding in the heifer farms studied (Table 4), except for the 1994 winter period in farm 3, where values were similar to the literature. Embryo losses are likely to occur during the first stages of pregnancy and may have contributed to an underestimate of the conception rates, considering the length of time post-breeding that pregnancy diagnosis was done. Kastelic et al. (1991), reported 6.1% embryonic death, as determined by transrectal ultrasound examinations, between days 24 and 40 (ovulation= 0). Another study with Holstein heifers found embryonic losses of 5.3%, as determined by the bovine pregnancy-specific protein B method, during the interval between initial diagnosis 30 to 45 days post-breeding and a subsequent evaluation at 60 days of gestation (Alexander et al. 1995).

Services per pregnancy were similar or better than the ones reported by Dolezel et al. (1985) (Table 3), except for the winter season of 1993 in farm 4.

Good overall conception rates were observed in both winter seasons (1993, 1994) in farm 3, while the proportion of heifers conceiving in both spring seasons seemed to be below the expectations (Table 3). This observation is consistent with the information reported by Stevenson et al. (1984) who found that first service conception in Kansas (US) was 81% in winter and 60% in spring. These authors suggest that winter advantages are associated with low temperatures and short daylengths. Embryo death may also have contributed to low spring fertility in farm 3. Potential embryo losses of 1.9% in winter and 4.4% in spring were observed between the ultrasound diagnosis on day 35 and the rectal palpation on day 75 in a group of heifers on farm 3 in 1994 (Table 5).

Data from farm 4 show a poor 1st service conception rate (Table 4) in the 1993 winter breeding season. Thirty percent of the heifers bred had to be inseminated more than once with poor conception rates at the 2nd and 3rd services (Table 4) resulting in 1.65 services per pregnancy (Table 3). Acceptable and varied overall conception rates were observed during the breeding seasons evaluated in farm 4. A large incidence of abortions was observed in heifers bred during the winter of 1993 with more acceptable values during the spring of the same year. In 1994, the proportion of abortions was low, however a similar seasonal trend was observed. The large number of heifers bred during the winter season in both years may have caused pasture shortages during the winter breeding periods, contributing to a large proportion of pregnancy losses after first palpation. The effect might be exacerbated during 1993 because of more adverse climatic conditions affecting pasture growth.

The seasonal comparison of pooled data from both years and both farms (Table 6) indicates similar overall conception rates for the winter and for the spring seasons. This result, may be the consequence of the poor winter performances and large numbers of heifers of farm 4 during both winter seasons neutralising the good winter performances of farm 3. The overall pregnancy rate was lower in the winter than in the spring because fewer heifers showed estrus in winter (Table 6). The results observed are consistent with the observations of Hurnik et al. (1975) who found that estrus occurs mainly at night. As heat detection is done with daylight, there are better chances of catching animals in heat in spring than in winter due to earlier observations in the mornings and later observations in the afternoons. Occasional pasture biomass shortages during the winter breeding period also may have contributed to the fewer animals observed in estrus. In some studies low levels of feeding in heifers have caused inhibition of estrus expression and cyclic ovarian function (Bond et al. 1958, Imakawa et al. 1983).

The percentage of heifers cycling and overall pregnancy rate were lower in 1993 than in 1994, however, overall conception rate was similar in both years (Table 7). Cause of a year effect can not be elucidated with the present data, but may be related to changes in pasture quality and availability.

The inadequate dairy herd breeding performances described by Cavestany (1992, 1993) were not confirmed by the results presented. However, data shows that the full potential was not achieved in some of the reported breeding seasons. Limitations to good performance in terms of proportion of cycling heifers, conception and pregnancy rates cannot be absolutely clarified from the available information. A better record system on the farms studied would have helped to clarify the reasons for such variations. Age at calving, lactation number, liveweight changes, body condition score and individual milk production are relevant missing data in this herd analysis, and generally absent from records of commercial Uruguayan farms. Better data related to feeding (i.e. pasture availability and quality) and climatic conditions as related to pasture production during breeding also would provide useful information related to seasonal and year to year fertility variations. Nevertheless, the possibility of a relationship with diet is not dismissed in the situations studied.

The reported diet for heifers (farms 3 and 4) was white clover-birdsfoot trefoil pasture in all situations. However, considering the diversity of factors that affect pasture dry matter (DM) yield and quality, a variable nutritional status of heifers in the different situations studied was likely to occur. Garcia (1996a) describes the DM yield of a typical cultivated pasture composed of white clover *(Trifolium Repens)*, birdfoot trefoil *(Lotus corniculatus)* and tall fesue *(Festuca arundinacea)* as 6,000; 9,000; 6,000 and 5,500 kg ha^{-1} for the first, second, third and fourth year, respectively. Diaz et al. (1996) conclude that the commonly used legumes in Uruguay (birdsfoot trefoil, white clover and red clover) produce 50% of the annual dry matter in spring, the remaining 50% being distributed in the summer, fall and winter. Leborgne (1984) established that good pasture digestibility is usually found in winter while lower values occur in summer, and that the grass/legume ratio and pasture management can also affect pasture quality.

In the case of the dairy farms (farms 1 and 2), a particular feeding management leading to low concentrations of BUN in one breeding season was associated with a good breeding performance. Many studies have reported a negative relationship between protein excesses in the diet and breeding performance in dairy cows (Claypool et al. 1980; Edwards et al. 1980; Ferguson and Chalupa 1989). Toxic effects of circulating urea and ammonia on gametes and early embryos have been mentioned as possible causes of reduced fertility. Reduced heifer performance in some of the breeding seasons described in this survey was partly related to failures in conception and embryo death. This kind of information has created some concern in Uruguay about the effect of feeding high protein pastures on fertility of dairy cows. Legume pastures have contributed to improved nutritional status and breeding performances of dairy herds in Uruguay, but on the other hand there is a local impression that adjustments to pasture utilisation still have to be done in order to get their maximum potential. The fact that in some situations, the high forage protein content may impair the reproductive strategy has created some confusion. It would be of a great value to clarify the actual incidence of the problem, and to determine which tools could be used to monitor and prevent eventual critical periods.

Conclusions

Information relative to management and breeding performance of two dairy farms and two heifer rearing farms was studied under common Uruguayan farming conditions. The full breeding potential, according to the literature, was not achieved in some of the situations reported. Poor first service conception rates leading to a higher number of services than expected to achieve acceptable overall conception rates were observed in the lactating herds under certain management practices. Failures in conception, embryo death and heat detection problems probably contributed for a large number of services per pregnancy and poor pregnancy rates of heifers in some of the breeding seasons studied.

The causes for the circumstantial reproductive problems cannot be elucidated from the data presented. Year and season effect cannot be fully evaluated because of the superimposed effect of each particular farm management practice. However, the information reported suggests possible negative effects of the diets associated to increased levels of BUN, winter pasture shortages, as well as negative seasonal effects associated to short days in winter.

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Manuscript 2

Breeding performance and blood metabolite concentrations of Holstein heifers grazing a legume pasture alone or supplemented with two levels of corn silage under Uruguayan management practices.

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Abstract

Two grazing trials and two intensive animal studies were done to evaluate the breeding performance and to assess blood metabolites of nulliparous Holstein heifers under three dietary treatments with different levels of crude protein (CP). Two hundred and sixty-two and 209 heifers from a co-operative rearing farm were utilised for a winter and a spring trial, respectively. A predominantly red clover *(Trifolium Pratense)* pasture was the basic component of the three diets during both seasons. Diet A represented pasture alone, while diets B and C represented pasture supplemented with corn silage at two levels 0.6 and 1.2%, and 1.2 and 2.0% of body weight dry matter basis for the winter and spring trials, respectively. Dietary CP (24.0% in winter and 28.0% in spring) was reduced ($P < 0.01$) 30% in winter and 53% in spring with high silage supplementation. Average daily gains (ADG) were similar and about 1 kg d^{-1} for heifers on treatments A and B during the winter and spring trials. Heifers on treatment C had lower ADG ($P < 0.01$, 0.7 kg day^{-1}) in both seasons. No effect of diet on the proportion of heifers detected in estrus and conception rates at 1st and 2nd services, was observed during the winter grazing trial. During the spring trial a lower proportion ($P < 0.01$) of heifers were detected in estrus and a lower ($P < 0.05$) first service conception rate was observed for heifers fed diet C. The overall conception rates were above 80% and similar among heifers fed the three diets in both seasons. Due to lower estrus detection, a lower pregnancy rate ($P < 0.05$) was also observed for heifers assigned to treatment C in the spring trial. Mean serum urea nitrogen was lower ($P < 0.01$) for heifers on treatment C relative to A in both trials. A lower serum progesterone level ($P < 0.05$ in winter, $P < 0.01$ in spring) was found in heifers fed diet A relative to heifers on diet C at the beginning of the luteal phase. A positive effect of the moderately high SUN on estrual behaviour, determining endocrine changes is hypothesised to explain the observed differences. It is concluded that impairing effects of high dietary CP on fertility observed in other studies do not apply in this case. Holstein heifers fed legume pasture and supplemented with moderate amounts of corn silage had a good and similar potential to become pregnant as herdmates grazing pasture only. However, if large amounts of silage are supplied special strategies for estrus detection should be considered.

Key words: Uruguay, dairy, breeding performance, heifers, legume pasture, corn silage, progesterone, serum urea nitrogen, prostaglandin

Abbreviation key: **ADF** = acid detergent fiber, **ADG**= average daily gain, **AI** = artificial insemination, **BCS** = body condition score, **BW** = body weight, **CARECO** = Campo de Recria Colonia, **CL** = corpus luteum, **CP** = crude protein, **DCP** = degradable crude protein, **DM** = dry matter, **DMI** = dry matter intake, **GLM** = general linear model, **INIA** = Instituto Nacional de Investigacion Agropecuaria, **LH** = luteinizing hormone, **LSM** = least square mean, **NEg** = net energy of gain, **NEl** = net energy of lactation, **NEm** = net energy of maintenance, **NDF** = neutral detergent fiber, **PG** = prostaglandin $F_2\alpha$, **SEM** = square error of the mean, **SUN** = serum urea nitrogen, **WSC** = water soluble carbohydrates.

Introduction

Uruguay is located in the South hemisphere within latitudes 30° to 35°. Climate is advantageous for pasture development and grazing on a year around basis. Cultivated pastures are currently utilised in the southwest portion of the country, where the best soils appear. They provide the basic diet in intensive farming systems like dairy and beef cattle fattening. Cultivated pastures have been directly related to the improvements in milk yield attained by the dairy industry in the last two decades.

Reproduction efficiency of Uruguayan dairy herds under grazing conditions is assumed to be acceptable, however, difficulties to impregnate cows and heifers have been observed in certain situations. Cavestany (1993) observed overall pregnancy rates, to be 71.1% and 58.2% in 1990 and 1991, respectively, in the dairy herd at La Estanzuela Experiment Station of The Instituto Nacional de Investigacion Agropecuaria (INIA). Lower breeding performance was observed in the same herd during spring services as compared to winter services, 69.8 vs 82.9% in 1990 and 40% vs. 62.5% in 1991 (Cavestany 1992). Pooled data from 1991 and 1992 of dairy heifers in the same Experiment Station indicate a pregnancy rate of 68% in winter and 40% in spring (Cavestany pers comm.). Qualitative aspects of local feeding strategies were pointed out as a possible impediment to breeding performance. Tosi and Wittenberg (manuscript 1) reported information relative to management and reproduction of two dairy farms and two heifer rearing farms in Uruguay. Lactating dairy herds utilising a legume pasture as their feeding regime had poorer first service conception rates (43.2 and 50.5%) than expected according to the literature, and a large number of services (2.20 and 2.23 services pregnant cow $^{-1}$) were needed to achieve acceptable pregnancy rates (82.2 and 83.8%). Supplementing pasture with corn silage and concentrate in one of the farms was associated with a 20%

improvement in first service conception rate and a reduction of 0.57 services per conception. In the same study, heifer herds managed under grazing conditions had a more acceptable breeding performance than cows, even though there were seasonal, annual and herd differences in fertility. It was speculated that lower breeding performance may be attributed to more than winter pasture shortages, and poor estrus detection during the short winter days, which are frequent situations in Uruguay. Impaired performances also might be related to some other factor when abundant pasture was offered. The fact that, even with no limitation on dry matter availability, the reproductive efficiency at farm level may be impaired has created some confusion among farmers and professionals involved in dairy production in Uruguay.

In order to contribute to a better understanding of the factors implicated in circumstantial reductions in fertility, this study approached the problem by evaluating animal breeding response to protein excesses relative to animal requirements for Uruguayan cultivated pastures.

The impact of dietary protein on fertility of high producing dairy cows has been considered as an important issue for many years. Most of the studies related to the subject have shown decreases in breeding performance of dairy cows and heifers when diets with a high percentage of crude protein (more than 20%) are fed (Claypool et al., 1980; Edwards et al., 1980; Ferguson and Chalupa, 1989; Elrod and Butler, 1993). There are, however, some reports that do not show that effect (Holtz et al. 1986; Howard et al. 1987). Ferguson and Chalupa (1989) reviewed the subject intensively. They proposed that the negative relationship between protein intake and fertility is better explained when excesses of degradable and undegradable protein related to requirements are considered as opposed to considering crude protein intake alone. They also indicate age, energy level of the diet and uterine health as important factors that can confound reproductive response to changes in protein levels. These authors mentioned that in most of the studies reviewed, the number of animals used (15 to 29 per treatment) was not adequate for an accurate evaluation of protein intake-fertility relationships. At least 80 to 100 animals per treatment are needed in order to detect 10% differences in conception rate (Ferguson and Chalupa, 1989).

A typical cultivated pasture in Uruguay has a high percentage of legumes, and often they are pure legume crops. Two of the most utilised species (white clover and red clover) can reach, in early vegetative stages, a content of 17% crude protein (CP) or more, plants (NRC 1989 Cozzolino et al. 1994). In addition, protein concentration under grazing conditions in this kind of pastures can easily exceed those levels when low grazing pressures occur. Heifer rearing farms usually designate these high quality pastures to feed animals prior to and during the service periods (winter and spring). Coincident with the spring service period, legume pastures are often the only component of the diet of lactating dairy cows. It is possible then that degradable protein excesses, under the described circumstances, can determine the presence of ruminal digestion byproducts at levels that can compromise breeding performance. Supplementation of legume pastures with corn silage is a commercially viable feeding strategy in Uruguay that would allow a reduction of dietary CP excesses while maintaining energy supply. Corn silage has a low concentration of CP (6.6%) with energy levels similar to those found in cultivated pastures (1.4 to 1.5 Mcal kg $^{-1}$ DM of NEl and NEm, and 0.90 Mcal kg $^{-1}$ DM of NEg) (Cozzolino et al. 1994).

This study was designed to obtain reproduction information for common management practices using relatively large numbers of animals representative of the major dairy production area of Uruguay. Nulliparous heifers from a co-operative rearing farm were selected to accomplish that purpose and to avoid the confounding effects of health status interacting with excessive dietary protein after calving reported in the literature (Barton et al. 1996, Carroll et al 1988, Howard et al. 1987).

The specific objectives of the study were: 1) to evaluate the breeding performance of Holstein heifers grazing a legume pasture supplemented with two levels of corn silage and compare it with that of heifers receiving pasture alone during the winter and spring service periods, and 2) to assess circulating levels of serum urea nitrogen and blood progesterone, and their relationship with eventual dietary effects on fertility.

Material and Methods

Two grazing trials were conducted in 1995 using a co-operative dairy heifer rearing farm (CARECO) located in one of the main dairy producing areas of southern Uruguay. Experimental periods started on May 29 and ended August 21 (85 d) for the winter grazing trial, and started on October 22 and ended January 12 (82 d), 1996 for the spring trial.

Nulliparous Holstein heifers were used in both trials. All heifers on the farm had to be in healthy condition, suspected to have reached puberty based on rectal palpation, with a body weight above 300 kg in winter and 250 kg in spring prior to being placed on test. Heifers diagnosed as nonpregnant and with a functional corpus luteum were classified as eligible for the study. Animals were assigned to dietary treatment on the basis of herd of origin, body weight and age. Age was estimated by considering the date and weight at admission to the co-operative farm, and dentition prior to treatment assignment.

The same legume pasture was used as the basic component of the diet in both trials. Seventy ha pasture was seeded with a mixture of white clover (*Trifolium repens),* at a rate of 1.5 kg ha $^{-1}$, and red clover (*Trifolium Pratense),* at a rate of 5 kg ha $^{-1}$ on May 1994. The pasture was fertilised at seeding with 80 kg ha $^{-1}$ of P_2O_5 and refertilised on March 1995 with 40 kg ha $^{-1}$ of P_2O_5. The pasture was not grazed for three months before starting the winter trial in order to get enough plant material for the purpose of the study. For the spring trial, pasture was rotationally grazed in such a way that approximately one month regrowth was available at the time grazing was initiated.

WINTER GRAZING TRIAL

The animals

Two hundred and sixty-two heifers from 32 farms were randomly assigned to dietary treatments on the basis of herd-weight-age group. An adjustment period to the diets was carried out from May 15 to May 28. Selected heifers were 21.3 ± .005 months old, weighed 342.5 ± 1.9 kg and had an average body condition score (BCS) (Garcia-Paloma 1990) of 2.09 ± .03 at the beginning of the adaptation period to the diets.

Heifer body weights and body condition score (scale 1 to 5) were measured on a weekly basis.

The treatments

Heifers assigned to treatment A received pasture as the sole component of the diet. Heifers assigned to treatments B and C received pasture supplemented with a low level and a high level of corn silage, respectively. Heifers on treatment B were offered corn silage at 0.6% BW, DM basis. Heifers on treatment C were offered at 1.2% BW, DM basis. The silage was harvested on February 1995 by chopping whole corn crop to a particle size of approximately 1 cm. Harvested corn forage was piled and packed prior to being sealed with plastic.

Pasture Design

The pasture was divided into three sections, with heifers assigned to each treatment grazing one of the three sections. Each section was subdivided into 11 paddocks. Two paddocks were utilised during the adaptation period to the diets. The remaining 9 paddocks were utilised during the experimental period. Heifers grazed each paddock for 7 days. The 9 paddocks utilised during the breeding period totalled 25.6, 21.2, and 14.5 ha for treatments A, B and C respectively. The stocking rates, during the same period, were 2.64, 3.31 and 4.74 heifers ha^{-1} for treatments A, B and C, respectively. Heifers assigned to treatments B and C were fed a weighed amount of corn silage once daily using a feeding area located adjacent to their pasture. Weighback from the feed bunks was weighed once a week after feeding. When orts were observed, feeders were cleaned up and the removed residue weighed. Silage feeding was done in the afternoon (12.00 to 16.00 h). A mineral supplement containing a maximum of 8% P, 12% Ca and 40% NaCl was supplied to all grazing heifers. One mobile mineral feeder per treatment was placed in the grazing paddocks. Mineral feeders were re-filled once a week at the time they were moved to the next paddock. All heifers had access to the mineral feeder. Heifers were offered pond water near the pasture twice a day, once in the morning and once in the afternoon. Distance to the pond averaged 600 m. Handling facilities for AI and blood sampling and sampling pens were beside the pond. All animal handling activities occurred immediately after they had an opportunity to drink.

One composite sample (10 sub-samples) representing pasture offered before grazing, and one composite sample (10 sub-samples) representing pasture refused after animals were removed from the paddock was taken weekly from each paddock. Each sub-sample represented a .24 m^2 quadrat and was cut approximately 0.02 m above the soil surface using shears. Weekly composite samples were brought to the laboratory immediately after being cut. Once in the laboratory, samples were immediately dried and ground. Dried ground samples were analysed for DM, CP degradable crude protein (DCP), ADF, NDF and ash. Two of the weekly composite samples (weeks 3 and 8) of the offered pasture were analysed for pasture WSC content. One composite sample, obtained by mixing all the weekly dried and ground composite samples of the offered pasture, was analysed for Ca, P, Cu, Mn and Zn content.

One composite sample (10 sub-samples) of corn silage offered to heifers was taken from feed bunks on a weekly basis. Since no nutrient determinations were done on refused silage, nutrient composition of silage weighbacks was considered similar to offered silage. Offered silage composite samples were handled and analysed in the same way as pasture composite samples. Silage pH was also determined in the laboratory. Pasture and silage sampling started the first week of the experimental period.

Reproduction management and measurements taken

Once the adaptation period ended, heifers were given two injections, each with 1 ml of prostaglandin; the injections being 11 days apart. The first PG injection was administrated on May 29 and the second one on June 9. Estrus synchronisation was done with the synthetic analogous of prostaglandin $F_2\alpha$ Glandinex, (400µg of 16-(chlorophenoxy)-w-tetranor-trans-Δ_2-PG $F_2\alpha$ per ml of Glandinex, Universal Lab Ltda., Montevideo, Uruguay, under license of ONO Pharmaceutical Co., Ltd., Osaka, Japan). A first round of six days (day 0 to day 5) of estrus detection was initiated the day heifers were injected with the second dose of prostaglandin (day 0= day of second injection). All inseminated heifers were observed for repeating estrus 17 to 24 days after 1^{st} service. All heifers repeating heat were inseminated again. No more than two inseminations were done to each heifer. All heifers not showing estrus during the second period of estrus detection were removed from the trial. Only heifers repeating estrus or showing estrus for the first time during the second period of estrus detection were maintained on pasture. A third round of heat detection and AI was done from day 34 to 53. Heifers not exhibiting estrus, and heifers exhibiting estrus for the third time during the third heat detection period were removed from the pasture on day 53. Five heifers were inseminated for the second time during the third heat detection period. These animals were held on their respective dietary treatments for an additional 24 days. Due to the low number of heifers remaining for this period, the daily routine was altered, in a way that three small pens were constructed on the pasture sections, one for each treatment. Water and weighed amounts of silage were provided in mobile feeders inside these pens.

Heifers were rounded up twice a day, at 7:30 h representing sunrise, and 1 hour before sunset at 17:00 h, and brought into pens, one per treatment, for estrus observation. Estrus was visually detected for one hour each time, heifers staying in the standing position after being mounted by their herdmates were considered in heat. All heifers observed to be in heat stayed in an insemination pen contiguous to the heat observation pens until they were inseminated. Heifers observed in heat in the afternoon were inseminated after morning heat detection of the following day while heifers in heat in the morning were inseminated before the afternoon heat detection of the same day. All heifers were bred by artificial insemination (AI) using the same technician using semen from one bull (Direction from Landmark Genetics, USA). Two rectal palpations were done, the first one 38 to 45 days after service, and the second one 90 to 120 days after service.

Coccygeal blood samples were taken for progesterone and urea determinations. Each inseminated heifer was sampled 10 to 14 days after first service for urea N determination and 21 to 22 days after every service for progesterone determination. Blood samples were drawn though a needle, dispensed into glass tubes and stored on ice. Within two hours after collection, serum was separated by centrifugation at 3000 x g for 10 minutes and stored in 1.5 ml plastic vials at –20 ° C until assayed. Blood progesterone data was used as one of the criteria for pregnancy diagnosis. For that purpose levels equal or above 5.0 ng ml^{-1} were considered as high progesterone, while levels below 5.0 ng ml^{-1} were considered as low progesterone

SPRING GRAZING TRIAL

The animals

Two hundred and nine heifers from 56 farms were selected for the spring trial. Three dietary treatments were assigned to heifers in a similar way to the winter grazing trial. An adjustment period to the diets was carried out from October 2 to October 21. Selected heifers were 21.2 ± .005 months old, weighed 281.2 ± .3 kg and had an average body condition score (BCS) of 1.66 ± .04 at the beginning of the adaptation period to the diets.

The treatments

Treatments were similar to the winter trial, except that heifers on treatment B were offered 1.2% of BW as corn silage DM basis, and heifers on treatment C were offered 2.0% BW as corn silage, DM basis. Corn silage used for the spring trial came from the same silo as was used for the winter trial.

Pasture Design

Pasture design was similar to that utilised during the winter grazing trial. However, during the spring trial each section was subdivided into 12 paddocks; utilising 3 for the adaptation period and the remaining 9 for the breeding period. The 9 paddocks utilised for each treatment during the breeding period totalled 24.0, 14.5, and 11.0 ha for treatments A, B and C, respectively. The stocking rates were 2.45, 4.16 and 5.24 heifers ha $^{-1}$ for treatments A, B and C, respectively. Silage handling was similar to the winter trial but with a different feeding time. Silage feeding was done from 20:30 to 5:30 h. Mineral supply, watering, estrus detection, and sampling routines were done in a similar way to the winter grazing trial.

Reproduction management and measurements taken

The breeding schedule was the same one utilised during the winter trial, differing only for timing of heat detection. In the spring trial, heifers were rounded up at 6.00 h and at 19.00 h. The estrus to breeding schedule was the same as in winter, thus breeding time was advanced 1.5 h in the morning and delayed 2 h in the afternoon. The first PG injection was done on October 22 and the second one on November 2. Blood sampling, handling, and storage until analysis were done in the same way as during the winter trial. Criteria used for pregnancy diagnosis using blood progesterone levels also were the same.

INTENSIVE ANIMAL STUDIES

Thirty of the heifers bred during the second period of AI in the winter grazing trial, and 30 of the heifers bred during the first period of AI during the spring grazing trial were selected for an extra sampling schedule. The group was created by selecting the first ten heifers exhibiting estrus for the first time within each treatment, during the specific AI round in both grazing trials. Heifers were maintained with the rest of treatment group but were sampled on a more frequent basis for blood progesterone and SUN. During the winter grazing trial 2 blood samples were taken every 2 days for a period of 22 days post first insemination. During the spring grazing trial blood samples were taken three times a week for 22 days after initial insemination. One blood sample was used for progesterone determination and the other one for urea N determination. Due to errors in heifer and sample identification, data from 1 heifer in winter and 1 heifer in spring were removed from the statistical analysis.

ANALYTICAL PROCEDURES

Dry matter determination was done by drying forage samples in a forced air oven (60EC) for 48 hours. After drying, samples were ground and subsampled for the rest of the analysis. Crude protein was determined as N x 6.25 with an automatic analyser (Tecator Kjeltec System 1030 Distilling unit, Tecator) according to the methods described by the Association of Official Analytical Chemists (AOAC, 1984). Protein degradability was determined using the alkaline protease from *Streptomyces griseus* in a method developed by Krishnamoorthy et al. (1983). Acid detergent fiber and NDF were determined according to the procedure described by Goering and Van Soest (1970). Ash was determined following the procedure described by the (AOAC 1984). Serum progesterone concentration was analysed by radioimmunoassay (Solid-phase 125 I radioimmunoassay kit, Coat-a-Count Progesterone; Diagnostic Products Corporation, Los Angeles CA.). In total 12 progesterone assays were run with an intra-assay coefficient of variation of 6% and an inter-assay coefficient of variation of 12%. Human serum was used to generate the standard curves which were validated for bovine serum. The equipment used to measure radioactivity was a EG and G Berthold gamma counter which automatically computed the progesterone concentrations by using the spline function. Samples and standards were assayed in duplicate, and the detection range of the assays was from 0.16 ng mL^{-1} to 40.0 ng mL^{-1}. Serum urea N was determined by hydrolysis of urea to ammonia by urease method (Uremia Kit Wiener Lab. Rosario Argentina). Water soluble carbohydrate concentration was determined by the procedure developed by Slominski et al. (1993).

A scale from 0 to 5 (1= very thin, 5= obese) with intermediate levels of 0.5, according to the methodology proposed by Garcia-Paloma (1990), was used to score body condition.

DATA ANALYSIS

Nutrient composition of complete diets (Table 3) was calculated as the sum of the amount of a nutrient supplied by the pasture plus the amount of the same nutrient supplied by the silage plus the amount of the same nutrient supplied by the mineral supplement, expressed as percentage of total DM intake. The estimation of the weekly offered and refused amount of pasture DM was calculated using the following formula:

kg pasture forage DM wk^{-1} (offered or refused) = (kg DM in composite sample/.24 m^2) X 10,000 m^2 X ha pasture

Weekly DM consumed was calculated by subtracting pasture forage DM refused from DM offered each week. Consumed amounts of CP, DCP, ADF and NDF per week were calculated in the same way as consumed DM. Three weeks of pasture data were eliminated due to errors in processing or analysis of forage samples.

Daily heifer pasture DMI was determined by the following formula: [(weekly DM consumed, kg)/(7 days)]/(number of heifers grazing the paddock during the considered week). Silage DMI was determined by the following formula: [(kg silage DM offered per week - kg silage DM refused per week)/(7 days)] / (number of heifers fed during the considered week). Mineral mix intake was calculated as the weight of the supplement placed into mineral feeders minus mineral mix remaining in each feeder at the end of each trial.

Linear regressions of weekly body weights (BW) on day of weighing, and of BCS on day of body condition scoring were done for each individual heifer. The b values representing the slope of the regression lines obtained were used as the estimation of individual BW changes (kg day^{-1}) and BCS changes (BCS unit day^{-1}).

Heifers were considered as cycling post estrus synchronisation when they were recorded as being bred during the trial. Heifers were considered as not cycling when they did not show estrus while participating in the trial. Pregnancy diagnosis was done using blood progesterone and rectal palpation data. Cycling heifers were considered pregnant when they met the following criteria: 1) the heifer did not repeat estrus after first or second service during their participation in the experiments, 2) the heifer had a high blood progesterone concentration 21-22 days after the last service considered, 3) the heifer had a positive pregnancy diagnosis at first palpation, 4) the heifer did not repeat estrus after the end of their participation in the experiment, and 5) the heifer had a positive pregnancy diagnosis at second palpation. In cases where the progesterone information was missing, heifers that met the other criteria were considered pregnant. Cycling heifers were considered open when at least one of the following conditions were observed: 1) the heifer showed estrus signs for a third time during their participation in the experiment, 2) the heifer had a negative pregnancy diagnosis at first palpation, 3) the heifer repeated estrus after the end of their participation in the experiment, and 4) the heifer had a negative pregnancy diagnosis at second palpation.

For the purposes of this study first service conception rate is defined as the percentage of heifers considered pregnant as a consequence of first service, relative to the total number of heifers cycling. Second service conception rate is defined as the percentage of heifers considered pregnant as a consequence of a second service, relative to the total number of heifers returning to estrus once after first service. Overall conception rate is defined as the percentage of heifers considered pregnant relative to the number of heifers cycling. Overall pregnancy rate is defined as the percentage heifers diagnosed pregnant relative to the total number of heifers in treated with PG.

STATISTICAL ANALYSIS

Grazing trials

Dry matter, CP, degradable CP, ADF, NDF, ASH, and water soluble carbohydrates contents of the pastures, silage and complete diets were analysed in a factorial arrangement as a complete randomised design (Steel and Torrie 1980) with sampling week as the experimental unit. The analysis was done by using the general linear models procedure (GLM) (Statistical Analysis System Institute, Inc. (SAS, 1985). The model for the variables analysed was $Y_i = \mu + W + T_i + WxT + \varepsilon_i$, where μ is the general mean, W is the week effect, T the trial effect and ε the random error. Values were expressed as least square means (LSM) ± standard error of the mean (SEM). Means separation was done by using the Bonferroni means separation test where $P < 0.05$.

Initial body weight, initial age, initial BCS of the three treatments and the effect of treatment on ADG, change in BCS, SUN and service intervals were analysed as a randomised complete block design (Steel and Torrie 1980) by using the GLM procedure (SAS 1985). The model for the variables analysed was $Y_{ij} = \mu + T_i + H_j + \varepsilon_{ij}$, where μ is the general mean, T is the treatment effect, H is the herd (block) effect and ε_{ij} the treatment-herd interaction used as error term. Values were expressed as LSM ± SEM. Means separation was done by using the Bonferroni means separation test (SAS 1985).

The effect of treatments on the proportions of heifers cycling and pregnant, that showed estrus within 6 days of prostaglandin treatment, and heifers that showed estrus after 6 days of prostaglandin treatment were analysed by proportions comparison using contingency tables and chi square analysis (Steel and Torrie 1980).

The effect of the three dietary treatments on initial weight and ADG in the presence or absence of estrus (cycling or not cycling, respectively) were analysed as a complete randomised design in a factorial arrangement by GLM procedure (SAS 1985). Means separation was done by Bonferroni means separation test. The model was $Y_{ij} = \mu + T_i + E_j + (T \times E)_{ij} + \varepsilon_{ij}$, where μ is the general mean, T is the treatment effect, E is the estrus effect, T x E is the interaction between treatment and estrus, and ε the random error.

Intensive animal studies

The initial weights and BCS of the three treatments, and the effect of treatments on ADG, changes in BCS, and blood progesterone within each sampling day were analysed as a complete randomised design by GLM procedure (SAS 1985). The model for the variables analysed was $Y_i = \mu + T_i + \varepsilon_i$, where μ is the general mean, T is the treatment effect, and ε the random error. Serum urea nitrogen and progesterone profiles were analysed as a complete randomised design in a factorial arrangement by GLM procedure (SAS 1985). The model was $Y_{ij} = \mu + T_i + D_j + (TxD)_{ij} + \varepsilon_{ij}$, where μ is the general mean, T is the treatment effect, D is the day effect, T x D is the interaction between treatment and day, and ε is the random error.

Results and Discussion

The daily mean, minimum and maximum temperatures during the winter trial were 9.5, 5.2, and 14.3 ° C, respectively. Spring trial daily mean, minimum and maximum temperatures were 19.2, 13.4 and 24.8 ° C, respectively. Total rainfall was 272 mm during the winter trial and 226 mm during the spring trial.

The legume stand prior to heifer placement on pasture was predominantly red clover with 10 % white clover and no weeds for both seasons evaluated.

Winter trial

Heifers assigned to treatment B were 8.3 and 6.7 kg lighter ($P < 0.01$) than heifers assigned to treatments C and A, respectively at the time the breeding period started (Table 8). When heifers were selected for trials from the farm herd, one month before the start of the adaptation period, average weights were similar for the three groups (314 ± 3.25, 314 ± 3.0, 313 ± 3.0 for treatments A, B and C respectively). This indicates a lower weight gain of heifers in treatment B in the period of time (1.5 months) between selection and start of breeding. The differences observed probably occurred during the two week adaptation period as a consequence of the low amount of silage supplied to heifers in treatment B and the need to restrict the offered pasture in order to encourage heifers to consume silage. Heifer weight, at initiation of the breeding period, in this trial (table 8) is above the minimum of 325 kg recommended for first breeding of Holstein heifers (Schmidt and Van Vleck, 1975). Heifer age at first breeding was significantly higher than the 15 months reported in the literature as optimum to minimise the length of the non-productive period of dairy females (Schmidt and Van Vleck, 1975; Johnsson, 1988). The increased age is related to low rates of gain obtained during rearing in Uruguay due to feeding strategies that usually confine heifers to areas of poor quality pasture. First estrus is

Table 8. Age, weight, body condition score (BCS), average daily gain, change in body condition score, and serum urea nitrogen (SUN) of Holstein heifers offered a high legume pasture forage alone or supplemented with varying levels of corn silage for winter and spring trials (LSM± SEM)

Treatment[x]	A	B	C	Treat P-value	Herd P-value
Winter trial					
n=	87	87	88	32 farms	
Initial age, mo	21.5±.07^{a}	21.2±.07^{b}	21.5±.07^{a}	.046	< ..001
Initial weight, kg	343.9± 1.8^{a}	337.2±1.8^{b}	345.5±1.7^{a}	.003	.001
Initial BCS	2.33 ±.05^{a}	1.92±.05^{b}	2.00±.05^{b}	< .001	.065
Avg. daily gain, kg	1.07± .06^{a}	1.03±.06^{a}	.74±.06^{b}	.007	.659
Change in BCS	-.05±.01^{c}	.06±.01^{a}	.01±.01^{b}	< .001	.610
SUN, mg dL^{-1} (n)	20.08 ±.92 (40)	17.24 ± .82 (49)	17.82±.83 (48)	.084	.934
Spring trial					
n=	69	71	69	56 farms	
Initial age, mo	21.09 ± .10	21.26 ± .10	21.26 ± .10	.449	< .001
Initial weight, kg	280 ± 1.4	283.7 ± 1.4	279.9 ± 1.4	.093	< .001
Initial BCS	1.65 ± .04	1.73 ± .04	1.60 ± .04	.090	.005
Avg. daily gain, kg	1.04 ± .03^{a}	.95 ± .06^{a}	.69 ± .04^{b}	< .001	.411
Change in BCS[y]	0.13 ± .01	.10 ± .01	.10 ± .01	.043	.643
SUN, mg dL^{-1} (n)	20.96± . 86 (55)^{a}	18.52 ± .94	13.28 ± 1.11 (38)^{b}	< .001	.325

[x] A= Pasture alone, B= Low silage, C= High silage

a, b, c LSMmeans, in the same row, with different letters are different ($p < .05$) as determined by Bonferroni means separation test.

[y] Differences between lsmeans were not detected using a Bonferroni means separation test.

not reached until the animal is 45 to 47% of mature weight, regardless of plane of nutrition (Crichton et al. 1960), therefore it is difficult to successfully breed Holstein heifers before 18 months of age under typical Uruguayan rearing conditions. Heifers assigned to treatment B were 0.25 months younger ($P < 0.05$) with respect to treatment C heifers, with heifers in treatment A being intermediate (Table 8). The differences observed in initial weight and initial age are minimal and probably not biologically relevant for the purposes of this study. Heifers on treatment A had better BCS at breeding ($P < 0.01$) as compared to heifers assigned to treatments B and C (Table 8). Body condition score was not measured at the time heifers were selected for the trial, thus it was not considered as a criterion to group heifers before treatment allocation. A general visual evaluation of heifers indicated that they were in a medium to low condition. Neutral detergent fiber and ADF content of pasture C (Table 9) are close to the values described by NRC (1989) for early bloom red clover (40% NDF and 31% ADF), which agrees with the actual maturity of the stand. Even though there were no statistical differences among treatments for ADF and NDF content (probably because of the large variability observed), fiber content of pastures A and B are below the levels of that described by NRC for early bloom red clover. These values may reflect the earlier stage of maturity of the pasture. In the present trial the grazing pressure determined that selective grazing occurred in the three pastures utilised, favouring the intake of leaves against stems. In the case of treatment C, a more severe pasture restriction than in treatments A and B determined a less selective behaviour. Therefore, considering that the nutrient content was calculated from estimations of consumed pasture, and not from whole plants, a more fibrous material was expected to be present in the pasture consumed by heifers assigned to treatment C. Crude protein content of the three pastures is higher than the value of 19.4% reported by NRC (1989) for early bloom red

Table 9. Nutrient composition of consumed pasture and silage, for winter and spring trials (LSM ± SEM)

Season	Winter (n= 6)				Spring (n= 9)			
	Pasture			Silage	Pasture			Silage
	A	B	C		A	B	C	
Dry matter %	21.55 ± 1.85	21.38 ± 1.85	24.90 ± 1.85	41.3 ± 2.23	25.05± 1.60	24.44 ± 1.60	25.76 ± 1.60	32.14 ± 1.87
PH				3.98 ± .04				4.02 ± .04
Nutrients, % dry matter basis								
Crude protein	23.96 ± 1.91	22.34 ± 1.91	21.73 ± 1.91	5.92 ± .23	24.92 ± 1.66	22.55 ± 1.56	22.74 ± 1.66	5.39 ± .19
Degradable CP [w], CP %	57 ± 5	58 ± 5	44 ± 5	73.1 ± ..50	52 ± 5	58 ± 5	52 ± 5	71.7 ± .40
Acid detergent fiber	13.63 ± 5.82	16.04 ± 5.82	25.05 ± 5.82	30.46 ± 1.10	11.08± 4.75	18.72 ± 4.75	11.72 ± 5.04	29.56 ± .92
Neutral detergent fiber	30.59 ± 6.14	26.13 ± 6.14	41.72 ± 6.14	68.77 ± 3.02	30.10± 5.01	36.09 ± 5.01	29.71 ± 5.01	72.13 ± 2.34
Ash[x]	9.6 ± .37*a*	9.37 ± .37*a*	8.36 ± .37*b*	6.48 ± .19	9.74 ± 3.0*a*	9.38 ± .30*a*	8.76 ± .30*b*	6.19 ± .16
Water Soluble CH_2O[y]	8.6 ± 1.44	8.11 ± 1.44	7.35 ± 1.44	10.00 ± 1.91	8.97 ± 1.44	8.36 ± 1.44	9.90 ± 1.44	9.36 ± 1.91
Ca[z]	.82	.72	.70	.26	1.07	1.24	1.20	.24
P[z]	.22	.23	.21	.10	.25	.21	.22	.11
Nutrients, mg kg^{-1}								
Cu[z]	15	10	14	3	15	13	16	6
Mn[z]	87	83	66	25	51	60	57	30
Zn[z]	27	19	26	9	25	25	30	7

[v] "n" is equal to the number of weeks analysed for each treatment. Week 1 was not included for calculations, since it was considered and adjustment period.

[w] CP= Crude Protein

[x] Ash was calculated from the offered pasture only .

[y] The number of samples used to claculate water soluble CH_2O was only 2 per treatment.

[z] Values were obtained from composite samples and are expressed as means for each treatment.

ab LSMeans, of pasture composition in the same row, with different letters are different (P= .0064) as determined by Bonferroni means separation test.

clover, which also reflects the selective grazing behaviour. Degradability of CP is lower than expected considering the value of 31% undegradable protein reported for red clover by NRC (1989). Whether the divergence observed is a true difference or is an underestimation of the actual degradability due to the analytical procedure is difficult to elucidate since no estimations for Uruguayan pastures are published. In the present study, degradability was determined by an in vitro procedure developed by Krishnamoorty et al. (1983) that simulates rumen proteolysis), while NRC reports estimations from in vivo and in situ studies. Although a high correlation coefficient of 0.61 between in vitro and in vivo procedures was found by Krishnamoorty et al. (1983), the absolute estimated values are not equivalent. Phosphorus and Ca content appear to be less than adequate for normal growth of the plant. Mills and Jones (1996) report that 2.00-2.60 and 0.28 - 0.60% are the optimum ranges of Ca and P content, respectively, for an optimum red clover growth in pre-bloom stage. Plant Ca and P concentrations in this trial are also below the values reported by NRC (1989 0.38 and 2.26 % for P and Ca, respectively) for vegetative or early bloom red clover. Low P concentrations in the plants are related to low availability of the nutrient in the soil (Brown 1970). The soils in the area where the study was conducted are low in available P and have a potential for a rapid fixation of the P added as fertiliser (Zamalvide 1996). Low Ca concentration is, however, unexpected considering that there is a generally adequate supply of this nutrient by Uruguayan soils (Moron and Beathgen, 1996). The Cu, Mn and Zn content of the pastures are similar to or greater than the values reported by NRC (1989; 9, 50 and 19 mg kg^{-1} for Cu, Mn and Zn, respectively), and within the sufficiency ranges for adequate plant growth reported by Mills and Jones (1996).

The pH of the silage utilised in this trial is below the recommended upper limit of 4.4 (Van Soest 1982) needed to guarantee the adequate preservation of the ensiled material. Silage DM and NDF contents are higher, and CP lower than the values reported by NRC (1989; 29, 53 and 8.4% for DM, NDF and CP, respectively) for corn silage with few ears. Pigurina (1991) did not find any difference in organic matter digestibility in corn silage samples from commercial Uruguayan farms across a wide range of DM contents (17.6 to 41.8%). Consistently silage ADF, which can be used as a predictor of digestibility (Schmid et al. 1975), corresponds to estimates provided by NRC (1989) for silage with 29% DM. Crude protein degradability is as high as expected (NRC 1989) considering the increase in soluble N compounds that takes place during the ensiling process (Bergen et al. 1974). Silage P and Ca content are lower than the values reported by NRC (1989). Similar considerations as discussed for pasture Ca and P concentrations can explain the differences observed. Copper, Mn and Zn content of corn silage reported in NRC (1989) feed composition tables is 10, 30 and 21 mg kg^{-1} respectively, these values are higher than the concentration observed in the silage of this study. The differences may be related to the plant maturity since there is a decrease in the concentration of most nutrients as a consequence of plant growth with the simultaneous relative increase of cell wall and storage compounds (Goñi 1996). The total DMI (Table 10) exceeded the amount established by NRC (1989) as the minimum requirement, based on heifer body weight (BW) and ADG. This finding is in agreement with the results obtained by Holden et al. (1994) who also found higher DM and NE_L intakes than the NRC (1989) recommendations with dairy cows grazing a grass pasture as the sole forage source. Holden suggested the discrepancy was due to an underestimation of energy requirements related to activity. Some adjustment for activity is reasonable for the current trial because animals were estimated to

Table 10. Dry matter intake (kg heifer $^{-1}$ day $^{-1}$) for each treatment in each experimental season (LSM± SEM)

Trial	Winter (n= 6)[x]			Spring (n= 9)		
Treatment	A	B	C	A	B	C
Pasture	10.8 ± 1..08	9.65 ± 1.08	7.88 ± 1.08	10.12 ± .88	8.97 ± .93	4.88 ± .93
Silage	-	2.12 ± .15	4.32 ± .15	-	3.52 ± .13	5.93 ± .13
Mineral[y]	0.051	0.050	0.056	0.047	0.048	0.053
Total	10.85 ± 1.07	11.82 ± 1.07	12.26± 1.07	10.17 ± .87	12.54 ± .93	10.86 ± .93

[x] "n" is equal to the number of weeks for each treatment. Week 1 was not included in values, it was considered and adjustment period.

[y] Mineral supplement contained a minimum of 8% P and 12 % Ca (n= 1).

walk 3 to 4 kmday^{-1} (NRC, 1989). On that basis, the DMI requirements are met by diet A in winter but are still exceeded by the rest of the diets evaluated. A lower energy concentration in feed than the NRC recommendation of (2.4 and 2.3 Mcal of ME/kg of DM for 300 and 360 kg dairy heifers respectively), or an underestimation of activity requirements as in Holden's study may explain some of the differences observed. Furthermore, increases in total DMI have been reported when dairy cows grazing high quality pastures were supplemented with corn silage (Rearte et al. 1990; Moran et al. 1990). Daily DMI in this study was estimated indirectly by measuring the weekly disappearance of pasture and silage DM, not allowing a precise statistical evaluation of individual intakes.

The nutrient composition of the complete diets is reported in Table 11. Reductions in CP content of 19% and 30% were achieved by supplementing pasture with the low and high proportions of silage, respectively. However, all three diets exceeded the CP and degradable CP recommendations of NRC (1989) (12.0% CP and 7.0% degradable intake protein for growing heifers). Supplementation with the large amount of silage determined a reduction of digestibility as inferred by the higher ADF content. Supplementation with the low amount of silage determined intermediate values of ADF. Neutral detergent fiber is above the minimum requirement (25%) established by NRC (1989) in the three diets. Mineral supply by the diets was more than adequate in the cases of Ca and Mn, borderline in the case of Cu and P and below the NRC (1989) recommendations in the case of Zn.

Heifers supplemented with the largest amount of silage had 30 % lower ADG ($P < 0.01$) than heifers on pasture only or fed the lesser amount of silage with pasture (Table 8). The lower digestibility of the high silage diet, as indicated by ADF, as compared with the other two diets, may have determined a restriction of the energy available for growth, which could not be equilibrated by a larger intake. Average daily gain of heifers on the low silage diet was similar to the pasture diet probably because of a larger DMI.

Small changes in BCS, as compared to those reported in the literature were observed in this trial (table 8). Frood and Croxton (1978) found a positive relationship of 25 ± 1.6 kg live weight gain per unit of condition score (0-9 scale). In this trial heifers gained approximately between 30 to 40 kg, however BCS changes were minimal. Heifers on pasture without corn silage supplementation decreased their BCS by .05± .01 units while heifers on treatments B and C increased their BCS in 0.06 ± 0.01 and 0.01 ± 0.01 units, respectively ($P < 0.01$). Body condition score has been described as a useful tool to assess body fat stores of dairy cows (Otto et al. 1991). However, Frood and Croxton (1978) found that the correlation between live weight and BCS during lactation was lower in first calving heifers than in mature cows. They presumed that the continued growth of heifers during lactation was the reason for a large variation in the data, and also observed that some heifers were gaining in live weight whilst actual body condition was falling. It is possible that the weight gained by heifers in the current study was composed of lean tissue and skeletal growth, resulting in the small changes observed. The differences in BCS change observed among treatments could be related to the energy availability of the diets and the initial BCS. According to NRC (1996) the energy required for a change in 1 unit score at a given body weight increases as BCS increase. Heifers assigned to treatment A had a larger ($P < 0.01$) initial BCS (table 8) than heifers on diets B and C, this determined a larger demand of energy to increase the score in a similar magnitude than in diets B and C. Assuming energy consumption by heifers

Table 11. Nutrient composition of total diets for each treatment, for each experimental season (LSM± SEM)

Season	Winter (n= 6)[v]			Spring (n= 9)		
Treatment	A	B	C	A	B	C
Dry matter %	21.41 ± 1.44*b*	25.26 ± 1.44*b*	30.10 ± 1.44*a*	24.40 ± 1.26	26.11 ± 1.26	29.24 ± 1.26
Nutrients, % dry matter basis						
Crude protein	23.96 ± 1.66*a*	19.36 ± 1.66*b*	16.63 ± 1.66*b*	24.92 ± 1.44 *a*	17.70 ± 1.35*b*	13.28 ± 1.44*b*
Degradable CP[w], CP %	57 ± 5	60 ± 4	55 ± 4	52 ± 4	62 ± 4	61 ± 4
Acid detergent fiber	13.63 ± 4.88*b*	18.75 ± 4.88*ab*	27.19 ± 4.88*a*	11.08 ± 3.98*b*	18.36 ± 3.98*ab*	21.23 ± 4.22*a*
Neutral detergent fiber	30.59 ± 4.89*b*	33.81 ± 4.89*b*	51.67 ± 4.89*a*	30.10 ± 3.99*b*	45.83 ± 3.99*a*	52.17 ± 3.99*a*
Ash[x]	9.60 ± .32*a*	8.83 ± .32*ab*	7.66 ± .32*b*	9.74 ± 26*a*	8.47 ± .26*b*	7.33 ± .26*c*
Water Soluble CH_2O[y]	8.60± 1.09	8.45 ± 1.09	8.29 ± 1.09	8.97 ± 1.09	8.64 ± 1.09	9.60 ± 1.09
Ca[z]	.88	.69	.60	1.13	1.01	.72
P[z]	.26	.24	.21	.25	.21	.20
Nutrients, mg kg^{-1}						
Cu[z]	15	9	10	15	11	11
Mn[z]	87	73	51	51	52	42
Zn[z]	27	17	20	25	24	12

[v] "n" is equal to the number of weeks analysed for each treatment. Week 1 was not included for calculations, since it was considered and adjustment period.

[w] CP= Crude Protein

[x] Ash was calculated from the offered pasture only .[y] The number of samples used to claculate water soluble CH_2O was only 2 per treatment.

[z] Values were obtained from composite samples and are expressed as means for each treatment.

a, b, c LSMeans , in the same row, with different letters are different (p= 0.01 for DM, CP, NDF and ASH, p= .0146 for ADF) , as determined by Bonferroni means separation test.

on diets A and B was similar, the difference in BCS observed between heifers in both diets ($P < 0.01$) could be related to the lower initial BCS of treatment B heifers. Heifers on treatment C probably had a similar demand of energy to increase their BCS as heifers on treatment B, since they had similar initial BCS, but the energy intake was lower inducing a lower ($P < 0.01$) increase in BCS.

Although heifers assigned the high silage supplemented diet gained less weight than the other two treatment groups, and had little change in BCS, the energy supplied by the diet during the trial should not have compromised breeding performance. The information reviewed suggests that only low levels of dietary energy and acute fasting leading to weight loss can inhibit estrus expression and cyclic ovarian function in heifers (Bond et al. 1958; Imakawa et al. 1983, 1984; McCann and Hansel 1986). In fact, Knuston and Allrich (1988) did not find alterations in estrus activity with a moderate feeding restriction that allowed a weight gain of 0.33 kg d^{-1}.

Breeding performance of heifers assigned the three diets (Table 12) was good and similar to that reported in the literature for nulliparous Holstein heifers fed normal levels of protein. The target for first service conception rate of dairy heifers has been reported as 77.8% 25 days after breeding (Kastelic et al. 1991) and 82% 45 days after breeding (Elrod and Butler 1993). First service conception rate was better in this trial than in the study by Elrod and Butler (1993) who found a 61% first service conception when excess protein (21.8% DM basis) was fed. Pregnancy and conception rate data obtained in this trial is similar to expectations under Uruguayan conditions, based on the ranges reported in a survey using information from two years on two heifer rearing farms in Uruguay (manuscript 1).

Table 12. Breeding performance of Holstein heifers offered a high legume pasture forage alone or supplemented with varying levels of corn silage during the winter and spring trials.

Treatment[v]	A	B	C	P-value
Winter trial				
Number of heifers treated with PG[w]	87	87	88	
Cycling heifers, % of treated[x]	89.7	86.2	81.8	.33
First service conception rate, % of cycling[x]	75.6	73.3	81.9	.44
Second service conception rate, % of returned[x]	52.9	50.0	66.7	.67
Service Interval (1st - 2nd), days	20.27± .67	20.25±.79	22.3±.71	.0844
Overall conception rate, % of cycling[x]	87.2	81.3	93.0	.10
Overall pregnancy rate, % of treated[x]	78.2	70.1	76.1	.44
Spring trial				
Number of heifers treated with PG[w]	69	71	69	
Cycling heifers, % of treated[x]	82.6 a	77.5 a	60.1 b	.007
First service conception rate, % of cycling[x]	73.7 a	69.1 a	50.0 b	.039
Second service conception rate, % of returned[x]	46.7	58.8	61.9	.64
Service Interval (1st - 2nd), days	18.77±.62	20.09±.68	20.28±.6	.1864
Overall conception rate, % of cycling[x]	86.0 a	87.3 b	81.0 b	.67
Overall pregnancy rate, % of treated[x]	71.0	67.6	49.3	.02

[v] A= Pasture alone, B= Low silage, C= High silage

[w] PG= Prostaglandin $F_{2\alpha}$

[X] P values are determined using chi-square analysis between treatments, df= 2

[Y] LSM±SEM

No effect of diet on breeding performance, as measured by the number of heifers cycling and conception rates at first and second service, was observed in the present trial (Table 12). Similar results were found when the breeding performance data was partitioned into heifers responding and not responding to PG treatment (table 13). These findings seem to be in disagreement with the reports reviewed by Ferguson and Chalupa (1989) in which breeding performance was negatively affected when high protein diets (17 - 20%DM basis) as compared to moderate protein diets (15 –16 % DM basis) were fed to dairy cows. However, these researchers postulated that the reductions in conception rate are more consistent with the excesses of undegradable and degradable protein intakes relative to the requirements than with the CP content of the diets.

Plasma urea nitrogen, which is positively associated with excesses of undegradable and degradable protein (Elrod et al. 1993, Elrod and Butler 1993), was found to adversely affect conception rate (Ferguson et al. 1993) and services per conception (Canfield et al. 1990) of dairy cows. The SUN concentrations in the present trial (table 8) were above the minimum of 15.4 mg dl $^{-1}$ (Folman et al. 1981) and below the maximum of 29.2 mg dl $^{-1}$ (Bruckental et al. 1989) reported in the literature reviewed for 20% CP diets. The observed values were below the average levels of 25.9 mg dl $^{-1}$ for dairy cows fed mainly legume pastures and slightly above the 16.5 mg dl $^{-1}$ of cows fed a pasture supplemented diet (corn silage/grain mixture) in a commercial Uruguayan farm (manuscript 1). Barton et al. (1996) found that SUN concentration were lower than Jersey cows and lower for primiparous than multiparous cows. The SUN peak levels after feeding found in Holstein heifers by Elrod and Butler (1993) were 17.5 and 23.6 mg dl $^{-1}$ for diets containing 15.45 and 21. 8% CP which are similar to results of the current trial. A tendency ($P < 0.10$) for lower SUN

Table 13. Breeding performance of Holstein heifers showing estrus within 6 days and after 6 days of Prostaglandin $F_{2\alpha}$ (PG) treatment, offered a high legume pasture forage alone or supplemented with varying levels of corn silage during the winter trial.

Treatment[x]	A	B	C	P-value[y]
Showing estrus within 6 days of PG treatment				
Winter trial				
Number of heifers treated with PG	87	87	88	
Cycling heifers, % of treated	64.4	50.6	60.2	.17
First service conception rate, % of cycling	78.6	68.2	77.4	.44
Second service conception rate, % of returned	27.2	45.5	63.6	.23
Spring trial				
Number of heifers treated with PG	69	71	69	
Cycling heifers, % of treated	63.8	60.6	55.1	.57
First service conception rate, % of cycling	68.2	67.4	52.6	.45
Second service conception rate, % of returned	50.0	57.1	61.1	.81
Showing estrus after 6 days of PG treatment				
Winter trial				
Cycling heifers, % of treated	25.3	35.6	21.6	.10
First service conception rate, % of cycling	68.2	80.6	94.7	.10
Second service conception rate, % of returned	100	100	100	
Spring trial				
Cycling heifers, % of treated	18.8	16.9	5.8	.06
First service conception rate, % of cycling	92.3	75.0	25.0	.022
Second service conception rate, % of returned	-	66.7	100	

[x] A= Pasture alone, B= Low silage, C= High silage [y] P values were determined using chi-square analysis between treatments, df= 2

levels was found in this trial for heifers fed the supplemented pastures as compared to the pasture alone diet. The absolute values of SUN levels were similar (treatment A) or below (treatments B and C) the limit of 20 mg dl $^{-1}$ reported by Ferguson et al. (1988) above which reduced conception rates can be expected in dairy cows. However, all SUN values in this trial were above 16 mg dl $^{-1}$ at which Elrod and Butler (1993) found reduced breeding performance of Holstein heifers.

Diet previous to the experimental period may have played a role in the breeding performance by affecting the number of heifers actually cycling in this trial. The higher ADG and lower initial weight of heifers not detected as cycling (Table 14) suggest the existence of heifers with a large compensatory growth within that group. Compensatory growth has been described as a period of more efficient growth following a period of environmental or nutritional stress (NRC 1996). The feeding restriction that those heifers might have tolerated could lead them to a situation close to the onset of anestrus previous to the start of the trials. Imakawa et al. (1984) observed that a weight loss of 0.111 kg d^{-1} or even a slight weight increase of 0.02 kg d^{-1} over 160 days resulted in anestrus of beef heifers previously cycling. The luteal secretion of progesterone and the estrous cycle length appear to be normal for the cycles immediately preceeding the onset of anestrus (Imakawa et al. 1983), therefore, it is probable that signs of an upcoming anestrus could not be properly detected in some of the heifers when corpus luteum assessment was done during selection of animals. Additionally, the accuracy of predicting a functional corpus luteum using rectal palpation was found to be between 79 to 80% (Archbald 1992, 1993). Adaptation to the experimental diets probably did not help to improve the energy intake, and some heifers could have

Table 14. Comparison of, initial weight, average daily gain (ADG) , of cycling and non-cycling Holstein heifers offered a high legume pasture forage alone or supplemented with varying levels of corn silage and for the winter and spring trial s (LSM±SEM).

Treatment[x]	A		B		C		P-value		
Cycling	No	yes	no	yes	no	yes	treat	cycle	t x c
Winter trial									
n=	9	78	12	75	16	72			
Initial weight, kg	324.67±10.01	346.60± 3.4	335.33± 8.67	337.81 ± 3.47	328.00±7.51	349.36±3.54	.9801	.0056	.2471
ADG, kg	1 .53 ± .19*a*	1.02 ± .06*d*	1.16 ± .16*ab*	1.01 ±.07*d*	.95±1.4*b*	.69±.07*e*	.0019	.0034	.3851
Spring trial									
n=	12	57	16	55	28	41			
Initial weight, kg	284.83±5.99	279.84± 2.77	273.81± 5.19	286.56 ± 2.82	271.18±3.92	285.9±3.24	.6596	.0289	.0481
ADG, kg	1 .28 ± .07*a*	.98 ± .03*d*	1.15 ± .06*ab*	.89 ± .03*d*	.76±.05*b*	.67±.07*e*	.0001	.0001	.0924

a,b,c LSMeans, in the same row for non-cycling animlas , with different letters are different (p< .01) as determined by Bonferroni means separation test.
d,e,f LSMeans, in the same row for cycling animals, with different letters are different (p< .01) as determined by Bonferroni means separation test.
[x] A= Pasture alone, B= Low silage, C= High silage.

initiated an anestrus period. Once animals adjusted to the increased energy intake at the beginning of the trials, the experimental period was probably not long enough to recover their cyclic activity. Re-initiation of estrus cycles occurred after 49 days of administration of a high energy diet to nutritionally induced anestrus heifers (Imakawa et al. 1986). The effect of an energy restriction on the estrous cycle previous to the initiation of the breeding period appears to affect all heifers in the same way regardless of treatment since no treatment x cyclicity interaction for ADG was observed (table 14).

Spring trial

Heifer initial weight, BCS and age were similar among treatments (Table 8). However, heifer initial weight and BCS were lower than at the beginning of the winter trial. There is usually a smaller pool of heifers available for spring service, due to the preference of farmers to have heifers calving in the fall rather in the early spring. This situation restricted the number of eligible heifers in such a way, that lighter heifers than usual had to be selected in order to get a reasonable number for the purposes of the trial.

Nutrient composition of the pastures are similar to that of the pastures on treatments A and B of the winter trial, except for Ca content which was higher, Mn content which was lower and CP content of diet A which was higher in this trial (Table 9). Similar considerations of maturity, selective grazing, and protein degradability as for the winter trial pastures apply for the pastures in this trial. Plant regrowth was utilised for the three treatments resulting in a similar stage of maturity across all pastures. Silage composition was almost identical to that of the winter trial, except for DM content, which was lower in the winter trial (Table 9). Since silage was made from the same crop, differences in DM probably reflect environmental conditions (i.e. humidity) at the time of chopping and compaction of the plant material.

The actual DMI of the three diets was higher than the minimum recommended by NRC (table 10). The low fiber content of diet A, as in the winter trial may explain its lower consumption with respect to B and C. Inversely to what happened in the winter trial, DMI of diet C seem to be lower than B, which is more consistent with the NDF content of the diets than was perceived in winter.

Reductions in CP content of 29% and 47% were achieved by supplementing pasture with the low and high amount of silage, respectively (Table 11). Diets A and B exceeded the CP and degradable CP recommendations of NRC for growing heifers (1989; 12% CP and 7.0% degradable intake protein). The CP and degradable protein content of diet C was very close to the recommended levels of the NRC (1989). As during the winter trial, supplementation with the highest amount of silage resulted in the highest amount of ADF while the amount supplied by lower amount of supplemental silage was intermediate relative to the pasture only diet (Table 11). The dietary mineral supply was adequate in all cases except for Zn. The high level of corn silage supplementation in diet C resulted in Zn intakes that were half the intake of the other two diets.

Heifers supplemented with the high level of silage had a 30% lower ADG ($P < 0.01$) than heifers offered pasture only or fed the lower level of silage supplement (Table 8). A lower digestibility of the high silage diet as compared with the other two diets was probably the reason for a restriction in energy availability for growth. The low silage diet had an intermediate digestibility and, as in the winter trial resulted in a higher DMI than the pasture alone diet. Changes in BCS were positive and similar among treatments (Table 8), which contrasts with the change in BCS found during the winter trial. All heifers in this trial had a lower initial BCS compared to the winter trial (Table 8), therefore lower energy is required to raise the BCS by one unit (NRC, 1996).

As was previously discussed for the winter trial, the ADG and BCS changes in the spring trial indicate an adequate energy intake during the experimental period, therefore, dietary energy should not have compromised the breeding performance. As for the winter trial dietary restrictions that may have occurred previous to the experiment, may have caused cycling heifers to gain less weight than non cycling heifers independent of the diet fed (Table 14). Consistent with observations for the winter trial heifers that were cycling were heavier than non cycling heifers in treatments B and C, but cycling and non cycling heifers on pasture had similar initial weights (Table 14). The three week adaptation period of spring allowed non cycling heifers of treatment A to catch up to cycling heifers at the beginning of the trial due to a higher rate of gain.

Overall conception rate was not affected by diet (Table 12). However, the proportion of heifers cycling and the proportion that became pregnant on first service were lowest ($P < 0.01$, $P < 0.05$, respectively) for heifers supplemented with the highest amount of silage (Table 12). The lower proportion of cycling heifers on diet C relative to A and B, seems to be independent of any possible carry over effect of the diet prior to the study, assuming the effect would have been similar for all heifers regardless of treatment.

The breeding performance data was partitioned into heifers responding to PG (first estrus observed within the first 6 days following the second PG injection), and heifers not responding to PG treatment (first estrus observed more than 6 days after the second PG injection) (Table 13). The adverse effect of diet C on cycling and first service conception rate, was more prevalent in heifers that did not respond to PG treatment. The high percentage of heifers at the estrual stage of the cycle while heat detection was carried out during the 6 days post PG treatment, may have contributed to override the problem. Helmer and Britt (1985) found that 85.8% of the attempted mounts on Holstein heifers was done by heifers during the preestrual and estrual stages of the estrus cycle, while heifers on the luteal stage of the cycle only accounted for 5.2% of the mounting attempts. Alexander et al. (1984) also found that mounting activity was low for heifers at midcycle and high during estrus. During the estrus detection periods after 6 days of PG treatment there were a large number of recently pregnant heifers, and a lower concentration of estrus per day than during the 6 days post PG treatment. This situation may have led to a lower mounting activity, contributing to a more difficult identification of the already, for some reason, reduced estrus expression of heifers on treatment C as compared to treatments A and B. The lower percentage of cycling heifers on treatment C resulted in a lower overall pregnancy rate (Table 12).

Serum urea nitrogen was reduced by high levels of corn silage supplementation for pastured heifers (Table 8), suggesting a more balanced energy to protein ratio than the pasture only diet. The conception rate results at first service of heifers on treatment C were inverse to what might be expected considering the dietary CP and SUN concentrations (Jordan and Swanson 1979a, 1979b; Ferguson et al. 1988; Ferguson and Chalupa 1989). Data from this trial is insufficient to give an adequate explanation to the impaired performance of heifers on diet C except for an eventual implication of very low dietary Zn (Table 11). A dietary level of Zn to optimise fertility has not been established, however, Georgievskii et al. (1981) mentioned that the amount of Zn deposited per kg weight gain in young fattened cattle is about 20 - 22 mg kg $^{-1}$. Zinc absorption by ruminants decrease with age, dairy calves can absorb 55% of intake, 5 - 12 months old calves 20% and cows

12% (Annenkov, 1981). Increased conception rates have been reported following Zn supplementation in cows, and higher calving rates (93%) were observed on Zn supplemented as compared to unsupplemented (62%) heifers (Piper and Spears, 1982, Nedyjlkov and Krustev, 1969 cited by Hurley and Doane, 1989).

Intensive animal studies

A similar body weight and BCS at the start of the breeding period were observed among treatments for both breeding trials (table 15). Changes in BCS for this selected group of cycling heifers, followed a similar trend as during winter and spring trials, with heifers assigned to treatment C having lower ADG (table 15). The similarity in animal response to diet for this reduced group of heifers with respect to that observed during the main trials validates the use of the data to interpret the physiological responses in the large scale trials.

Mean SUN of 11 samples taken during one estrus cycle were lower ($P < 0.01$) for both silage/pasture diets relative to the pasture alone diet in the winter trial. In the spring trial, the SUN was lower ($P < 0.01$) for heifers on the high silage diet relative to the heifers on the pasture diet, while heifers on the low silage treatment had intermediate values (Table 15). Factorial analysis of the plasma progesterone profiles indicate an effect ($P < 0.01$) of day of sampling on progesterone level, but not of dietary treatment. No interaction between day of sampling and treatment was detected. These results suggest a similar effect of sampling day on the progesterone level regardless of the dietary treatments. When the effect of treatment on serum progesterone concentration was tested separately for each day of sampling, a lower level was observed for heifers fed diet A relative to those fed diets B and C on day 2 in the winter trial ($P < 0.05$), and for heifers fed diets A and B as compared to C on day 3 in the spring trial ($P < 0.01$) (Table 15). These results

Table 15. Weight , average daily gain (ADG), body condition score (BCS), change in body condition score, serum urea nitrogen (SUN), serum progesterone (P_4), and breeding performance of a subgroup of Holstein heifers serially sampled, offered a high legume pasture forage alone or supplemented with varying levels of corn silage for winter and spring trials.

Treatment[x]	A	B	C	P-value
Winter				
n=	9	10	10	
Weight start of profiles, kg	373.00±10.09	368.60±9.57	399.80±9.57	.2650
ADG, kg	.92±.06*a*	1.06±.06*a*	.68±.06*b*	.0002
BCS start of profiles	2.11±14	2.20±13	2.20±13	.6720
Change in BCS	-.05±02*b*	.04±02*a*	.02±02*ab*	.0332
Mean SUN, mg dL^{-1} [y]	21.38±58*a*	18.09±.57*b*	16.16±.57*b*	.0001
P_4, ng mL^{-1}				
Day 0 after service	.157±04	.157±05	.377±18	.1844
Day 2 after service	.183±03*a*	.615±29*b*	.557±17*b*	.0461
Day 14 after service	8.63±1.24	8.63±76	7.38±1.24	.6630
Spring				
n=	9	10	10	
Weight start of profiles, kg	340.20 ± 10.09	335.10 ± 9.57	334.80 ± 9.57	.2650
ADG, kg[z]	.83±06	.78±06	.63±06	.0493
BCS start of profiles	1.94±14	2.10±13	1.95±13	.6720
Change in BCS	.12±03	11±02	.07±02	.3383
Mean SUN, mg dL^{-1} [y]	18.49±58*a*	13.14±54*ab*	11.87±54*b*	.0001
P_4, ng mL^{-1}				
Day 0 after service	.14±06	.13±04	.20±07	.6129
Day 3 after service	.56±07*a*	.58±.5*a*	1.06±17*b*	.0084
Day 14 after service	9.00±97	7.80±30	7.28±38	.1152

[x] A= Pasture alone, B= Low silage, C= High silage

[y] Number of SUN sample days for each heifer was 11

a,b,c LSMeans, in the same row, with different letters are different ($p < .05$) as determined by a Bonferroni means separation test.

[z] Differences between lsmeans were not detected using a Bonferroni means separation test.

partially agree with some studies that found a decrease in serum progesterone (Jordan et al. 1983; Jordan and Swanson 1979b) when cows were fed high (> 19%) as compared to moderate to low (< 16.3%) CP diets. However, these researchers found the differences later in the estrous cycle (14 to 15 days after estrus). Also, their trial was opposite to the current trial in that reduced fertility was observed for cows fed the high CP diet (Jordan and Swanson 1979b). Elrod and Butler (1993) did not find differences in progesterone concentration during the estrous cycle of dairy heifers fed either high (21.8%) or low (15.45%) CP diets. Carroll et al. (1988) only found reduced plasma progesterone concentrations when a high CP diet was fed to cows with reproductive disorders. In the present trial the comparisons done at 0, 4, 6, 8 10, 12 14, and 16 days after breeding in winter and 0, 5, 7, 10, 14, and 17 days after breeding in spring did not show any effect of dietary treatment on serum progesterone.

The reduced plasma progesterone concentrations in treatment A shortly after breeding are coincident with increased SUN levels during the cycle relative to diets B and C in the winter trial diet and C in the spring trial. Based on in vitro urea-N inhibition of LH receptors in the corpus luteum (Haour et al. 1974), Jordan et al. (1983) hypothesised that high endogenous SUN could cause an inhibition of LH binding to its receptors in the corpus luteum as well. This may lead to a decrease in plasma progesterone concentrations, which may explain the results observed in the current trial. The effect however disappeared on days 4 to 5 and similar profiles were observed for the remainder of the estrous cycle.

The unexpected lower proportion of cycling heifers and the reduced first service conception rate detected on treatment C in the large scale spring trial may be have been due to endocrine changes that occurred during estrus. Presence of a corpus luteum during the diestrus or luteal phase of the estrous cycle results in high blood progesterone concentrations. The lysis of the corpus luteum by prostaglandins leads to a decrease in blood progesterone and to the growth of the ovulatory follicle (Quirk et al. 1986). In turn estradiol is produced by the follicle and is reported to be the endogenous hormone responsible of estrus induction in cows and heifers (Cook et al. 1986; Coe et al. 1988; Stewart and Stevenson 1991). However, a prerequisite to the behavioural expression of estrus, as induced by estradiol, is a very low basal level of plasma progesterone (Lemon et al. 1975). Estradiol is also involved in a discharge of LH from the pituitary gland that occurs simultaneously to the estrus period (Lemon et al. 1975) leading to ovulation. Schams et al. (1977) reported concentrations between 0.2 and 0.6 ng ml^{-1} plasma progesterone for dairy cows from two days pre- to three days post- ovulation. Once ovulation has occurred a new corpus luteum develops on the ovary raising the progesterone concentrations and starting a new luteal phase. Exogenous progesterone has been reported to clearly inhibit estrus behaviour even with the existence of estrus-inducing concentrations of estradiol (Rajamahendran et al. 1979, Vailes et al. 1992) and to prevent the preovulatory surge of LH (Lee et al. 1988).

The progesterone mean values obtained on day 0 for treatments A and B in the present trial were below the detection limit of the assays (0.16 ng ml^{-1}). In fact 20 of the 53 heifers sampled for progesterone determination on day 0 during both seasons had non detectable values (0 for statistical analysis), and 9 others had values between 0.10 and 0.15. Considering this situation, the confidence of the values obtained and the statistical assessment of plasma progesterone on the service day may be questionable. The observed dietary effect on progesterone suggests that the basal levels on day 0 were similar among treatments and remained low for a longer time for animals on treatment A in the winter trial and on treatments A and B in the spring trial. A second explanation might be that levels were already different during the estrual phase remaining that way until days 2 to 3 after service. A similar blocking effect of LH receptors in the CL as a consequence of high SUN (Jordan et al. 1983) might have occurred before luteolysis as well, and the differences detected in this trial were the end of a period of low progesterone on high SUN heifers. We failed to detect reduced progesterone levels at the time of an expected luteolyisis (15 to 17 days after estrus) on the heifers fed the high protein diet. This may be explained by the fact that progesterone profiles in the present trial were done on bred heifers, 46 of which became pregnant out of 58 tested during both seasons. Therefore, the data obtained does not represent a normal complete cycle without pregnancy, not allowing a proper assessment of any difference that may have occurred during the onset of luteolysis.

Heifers assigned to treatment C in both seasons during the current trial could be in a similar situation of that reported by Duchens et al. (1995a). These researchers detected an effect of suprabasal levels of progesterone after luteolysis on the pattern of follicular growth, estrus behaviour and time of ovulation in heifers. Suprabasal levels of progesterone were defined by Duchens et al. (1995a) as the concentration range between the maximum plasma level at which ovulation normally occurs and the minimum progesterone concentration at which ovulation is completely blocked. These researchers identified this range to be from 0.25 to 0.63 ng ml^{-1} in their trial. The mean SUN for heifers on treatment C was within that range in the winter trial and very close to the lower limit in the spring trial. In the Duchens study, increased progesterone within the determined range resulted in signs of estrus becoming progressively weaker in intensity and longer in duration; and most of the heifers within the range did not stand to be mounted. On the upper limit of the range, ovulation was suppressed in one heifer; none of the heifers stood to be mounted and only weak secondary signs of estrus were observed. Other researchers also found that standing to be mounted was the estrus behaviour trait most sensitive to progesterone inhibition (Davidge et al 1987). Ovulation is retarded as a consequence of suprabasal progesterone levels and conception rate is impaired (Duchens et al. 1995b). These researchers speculate that suprabasal progesterone concentrations can be high enough to block the positive feedback of estradiol on the release of gonadotropin-releasing hormone with a subsequent delay of the LH peak and subsequent ovulation. Thus, high basal progesterone levels may affect fertility in two ways: an early insemination relative to ovulation time; and a prolonged growth of the ovulatory follicle that may impair the normal maturation of the oocyte.

It may be hypothesised that heifers fed the high silage diet underwent a situation in which progesterone levels were slightly higher than in heifers fed the unsupplemented pasture during the periovulatory period. That situation might be responsible for the poorer estrus detection and first service conception rates observed on diet C during the spring trial. The length of the estrus cycle (1st to 2nd estrus) in repeating heifers was similar among treatments during both large- scale trials (Table 12). This is not consistent with Duchens (1995a) who found that the interovulatory interval was longer (26 to 27.8 days) in suprabasal progesterone heifers. During the winter trial a tendency ($P < 0.10$) toward a shorter cycle length was observed in treatment A, and during the spring trial, although statistical differences were not detected, the lowest value also corresponded to treatment A.

The SUN ($P < 0.01$) of heifers on diets B and C was lower than A during winter (Table 15) are consistent with the trends ($P < 0.10$) observed during the large winter trial (Table 11). Nevertheless, the differences in estrus detection and breeding performance observed in the spring trial were not confirmed during the winter trial. The inconsistencies might be related to higher SUN levels of heifers on the high silage diet during the winter trial than those found during the spring trial. In addition, the decreases in SUN related to high levels of corn silage supplementation during the spring trial were similar in the large and small scale assessments (37 and 36% respectively), while the reduction observed in the winter large scale trial was lower than in the smaller scale trial (11% and 24%, respectively). It might be that there were no differences in progesterone basal concentrations during the large winter trial.

Duchens et al. (1995a) stated that the changes in estrus behaviour and ovulation of heifers with suprabasal progesterone levels are similar to those reported in repeat breeders, and suggest that one cause for the observed changes may be related to incomplete luteolysis leading to increased progesterone levels at the time of estrus. It is uncertain whether silage supplementation through some unknown factor determined an incomplete luteolysis, or the presence of larger SUN levels on heifers fed the sole pasture diet enhanced a more complete luteolysis determining low progesterone basal levels. The latter hypothesis is in accordance to the observations described in a review by Butler (1998) regarding an increased secretion of PG by bovine cultured endometrial cells in response to increasing urea concentrations. It may also be hypothesised that low blood progesterone might have occurred at the onset of luteolysis because of the blocking effect of SUN on CL receptors as speculated by Jordan et al. (1983). If that was the case, a less inhibitory effect of progesterone on oxitocin uterine receptors may have enhanced the role of estradiol on luteolysis. Estradiol is necessary to initiate the changes in oxitocin receptors in the uterus and serum concentrations of PG that precede luteolysis (McCracken et al 1984).

The moderately high SUN concentrations observed in heifers fed legume pastures in this study were probably not high enough to cause reductions in breeding performance as was reported in other experiments. It is proposed, instead, that the pasture only diet exerted a positive effect on estrual behaviour, possibly through endocrine changes that may result in better breeding performance. This effect may be lessened when estrus detection is facilitated by having a large proportion of animals on the proestrual and estrual phases at the time of estrus detection.

Conclusions

The impairing effects on fertility of high CP diets reported in the literature do not seem to apply for the case of nulliparous dairy heifers fed on pure legume pastures under Uruguayan grazing conditions. The supplementation of a legume pasture with two levels of corn silage did not result in any change of conception rate of heifers given two AI chances in each of the two breeding seasons evaluated (winter and spring). However, the supplementation with a large amount of corn silage (2.0% of BW) during the spring services resulted in poorer estrus detection and first service conception rate lower than silage unsupplemented or intermediate supplemented groups. It is hypothesised that the difference between seasons was related to the lack of a dietary effect of silage on SUN during winter, and the favourable effect that moderately high SUN may exert on endocrine events that induce clear estrus behaviour. Nevertheless, a negative effect of dietary mineral deficiencies, especially Zn, is not dismissed.

The utilisation of corn silage to supplement legume pastures is a useful tool widely utilised in Uruguay either to overcome periods of pasture shortages or to increase stocking rates. This study evidenced that bred dairy heifers fed legume pastures supplemented with moderate amounts of corn silage had a similar potential to become pregnant as herdmates fed on a pasture only diet. However, if large amounts of silage are supplied as supplement care must be taken regarding estrus detection strategies to achieve high breeding performances.

Further research on more precise hormonal assessments during the estrus cycle, and its association with estrus behaviour and fertility under Uruguayan common feeding strategies, will contribute to a better understanding of the mechanisms involved in the variable reproductive performance of dairy cattle. A study of the relationship between the trace mineral content of feeds commonly used in Uruguay for ruminant diets and reproductive performance of dairy cattle is also recommended.

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GENERAL DISCUSSION

Milk production in Uruguay depends on a grazing farming system. Annual grazing crops and legume pastures are planted on a rotational basis to achieve high milk yields per hectare. Dairy cattle breeding performance under those circumstances have been acceptable but variable among seasons and years. There is no precise documentation of the magnitude of those variations and the factors that may determine such behaviour. This study attempted to define the problem by analyzing two years of records of breeding performance available on 4 large commercial farms. This study also determined the effect of feeding corn silage to supplement the high protein (>17%) legume pasture diet commonly utilized in Uruguay, on SUN and breeding performance of heifers

Breeding performance under current production practices

Dairy Cows

Management and breeding data from two commercial dairy farms, located within the main dairy producing area in Uruguay were collected.

Breeding management was according to the usual recommendations in Uruguay. One extended breeding period per year was done on both farms with the seasons selected for the breeding periods (winter, spring) being consistent with a classical study done by Faggi et al. (1978) who observed that best milk yields were obtained with cows calving in the fall and winter. Estrus was visually detected twice a day, early in the morning and late in the afternoon. Artificial insemination was done approximately

12 hours after heat detection with several bulls of proven fertility per farm. Cows feeding management was representative of the most common situations in the country. For both farms, cows were pastured on legume stands of white clover, red clover and birdsfoot trefoil during the breeding periods studied in both farms.

The breeding data, under the pasture feeding regime, indicated that first service and overall conception rate were poorer than targets recommended in the literature (Esslemont, 1992; Morrow, 1980). Although conception rates were higher, services per conception were also higher than those reported in the literature for similar conception rates (Wattiaux, 1996; Esslemont, 1992). However, the herd grazing a similar pasture supplemented with corn silage and cereal grains had better conceptions and less services per conception than the values reported in the literature (Wattiaux, 1996; Esslemont, 1992; Morrow, 1980 a). Blood urea nitrogen levels of the supplemented cows were found to be lower than those on the pasture only diet. The better breeding performance of low BUN cows is consistent with the information reported in several studies (Ferguson et al. 1988, Canfield et al. 1990; Ferguson et al. 1993; Butler et. al. 1996), suggesting that pasture may affect fertility by increasing concentrations of urea in blood.

Dairy heifers

The two heifer rearing farms had two different breeding seasons were done per year, one in winter and one in spring. Artificial insemination strategies were based on estrus synchronisation, while estrus detection and subsequent insemination were conducted similarly to the dairy cows. On both farms, heifers grazed cultivated legume pastures (white clover-birdsfoot trefoil) on both heifer-rearing farms evaluated.

Consistently with the literature (Stevenson et al., 1983; Butler and Smith, 1989), conception and pregnancy rates were generally better for heifers than cows. However, lower first service conception rates than those reported in some studies with heifers (Kastelic et al., 1991, Elrod and Butler, 1993) were observed in most of the seasons evaluated. The differences may have been due in part to the longer time between service and pregnancy diagnosis in this study, and the embryo losses that are likely to occur during the first stages of pregnancy (Kastelic et al.,1991; Alexander et al. 1995). Services per pregnancy were similar to those target values reported for heifers by Dolezel et al. (1985) except for one winter breeding season in one of the farms.

Conception and pregnancy rates varied with farm, season and year in this study. For one of the farms, better results were obtained in winter relative to spring breeding, because of better conception rates at first and at second services. Embryo losses were likely to have occurred to a larger extent during spring relative to winter on this farm, probably contributing to the differential response. Conversely, on the other farm better results were observed in the spring breeding relative to the winter breeding. The poorer pregnancies and conception rates during winter were probably due to pasture shortages induced by a high stocking rate.

Pooled data from both farms indicate similar conception rates in both seasons. However, pregnancy rate was lower in the winter than in the spring, because fewer heifers were detected in estrus. Since estrus occurs mainly at night (Hurnik et al. 1975), poorer estrus detection in winter may have been due to later observations in the mornings and earlier observations in the afternoons due to longer nights. Changes in pasture quality and availability may have also caused a better estrus detection and a better pregnancy rate during 1994 than during 1993.

Legume pastures and heifer fertility

The information obtained from the farms surveyed indicate that a variable response on breeding performance of Uruguayan dairy herds actually exists and that many problems may be underlying it. Pasture shortages during winter, and poor estrus detection are frequent and certainly contribute to impaired fertility. However, there is evidence showing that even when abundant pasture is offered reduced conception rate may be observed, and that the excessive CP content of the legumes utilised may be implicated. Several studies have approached the problem of excess dietary protein on fertility of dairy cows (Claypool et al. 1980; Edwards et al. 1980; Holtz et al. 1986; Howard et al 1987; Ferguson and Chalupa 1989) indicating a negative relationship between fertility and BUN. Dietary energy, age and uterine health after calving are factors interacting with dietary protein and its effect on fertility (Ferguson and Chalupa, 1989). Therefore, it was decided to investigate the problem by using similar age nulliparous dairy heifers to eliminate possible confounding effects of calving on pregnancy and conception rates, and to contribute to the scarce information relative to heifers. Furthermore, the opportunity of utilising a large number of animals as were available at a heifer rearing farm and animals representative of a large number of herds from a large dairy producing area, favoured an accurate assessment of the problem.

The breeding performance was evaluated under the same management conditions utilised in commercial situations during two grazing trials (one in winter and one in spring). A typical legume pasture that would result in high CP intake (24 and

25% DM basis) was selected as the basic diet, while corn silage was used at two levels during each season to supplement pasture in an attempt to reduce CP intake and circulating urea. The pasture diet resulted in moderate circulating levels of urea (20 to 21), as measured by SUN, when compared to the critical values to affect fertility reported in the literature (> 20 ng dL^{-1} , Ferguson et al., 1988; Elrod and Butler, 1993). Silage supplementation reduced CP intake and SUN, but CP and degradable CP intake still exceeded requirements (NRC, 1989). Average daily gain and BCS measured during the trials indicated that energy supply was adequate and probably did not affected the breeding performance of heifers.

Overall conception, was not affected by diet, and was consistent with those reported in the literature (Kastelic et. al., 1991 and Elrod and Butler, 1993). However, first service conception rate and the percentage of heifers observed in heat was reduced when the largest amount of silage (2.0% BW, DM basis) was offered during the spring grazing trial. As a consequence of the lower proportion of heifers showing estrus, the pregnancy rate, as a percentage of total heifers, was also reduced.

The concentration of Zn in the high silage diet was below recommendations for heifers (NRC, 1989) and may have also contributed to poor breeding response. Zinc supplementation has been found to increase conception rate in heifers (Hurley and Doane, 1989).

The dietary effect observed during the spring trial could also could be associated with endocrine changes that might have occurred during the periovulatory period. The assessment of blood progesterone levels on a subsample of heifers from the grazing trials showed that a progesterone concentrations were lower at the beginning of the luteal phase for heifers fed pasture only than the high silage supplemented pasture diet. The lower progesterone concentration was associated with higher SUN, which has been reported in other studies (Jordan et. al. 1983; Jordan and Swanson, 1979b), but at different stages of the estrous cycle. Because most heifers serially sampled became pregnant keeping high blood progesterone concentrations after peak levels, and the analytical procedure for progesterone determination was not accurate at basal concentrations during estrus, progesterone concentration at the onset of luteolysis and during the estrus phase could not be properly assessed. However, if progesterone concentrations during estrus followed the same trend observed at the beginning of the luteal phase, poorer estrual behaviour on high silage supplemented pastures may be attributed to elevated suprabasal progesterone levels which were shown to determine weak estrus signs and poor conception rates (Duchens et al. 1995a, 1995b). The mechanisms linking SUN concentrations with blood progesterone are not clear, but some studies have reported increased prostaglandin secretion by endometrial cells in response to increased blood urea concentrations (Butler, 1998) which may render a more complete luteolysis leading to low basal progesterone levels. Others speculated a reduced responsiveness of the corpus luteum receptors to LH because of high SUN with a consequent reduction in progesterone production. (Jordan et. al. 1983)

In the present study, there was not a dietary response in terms of fertility of bred heifers fed diets with different levels of CP after two chances of AI. Opposite to what was expected high protein legume pastures may induce a positive effect on overall herd pregnancy rate by enhancing estrus behaviour, through moderately high SUN levels.

General conclusions

1) Compilation of management and breeding data form Uruguayan commercial dairy farms indicate that seasonal and year variation in pregnancy rates does exist and that, among other factors (day length, seasonal variation in pasture quality, stocking rate) differences could be associated to the high CP content of the legume pastures commonly used to feed dairy cows and heifers.
2) The experimental evaluation of a high CP legume pasture under Uruguayan grazing conditions did not result in extremely high concentrations of SUN, and did not reduce pregnancy and conception rates of nulliparous dairy heifers.
3) According to the results of this study, if a negative effect of Uruguayan legume pastures on fertility does exist it might be the result of interactions with other factors that were absent in the animals included in this experiment.
4) Moderate amounts of corn silage can supplement legume pastures during breeding without affecting dairy heifers fertility. However, corn silage, supplied at 2.0 % of BW (DM basis), reduced first service conception and the proportion of heifers that showed estrus behaviour.

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APPENDIX 1. Services per bull as % of total inseminations of two commercial dairy farms. Manuscript 1.

	Farm 1		Farm 2
Bull name	1994	1995	1994
Dylan	4.1	4.5	
Rocky	2.2		
Tong	5.2		
Viscoun	0.9		
Sailor	0.7		
Ari	0.7	20.1	
Eclain	6.6		
Freso	6.4	7.2	
Perry	3.9	0.6	
Richard	11.4	2.0	
Neutrad	4.6		
Raboner	3.4		
Magister	5.2	0.8	
Delight	3.2		
Bull (no AI)	15.2	0.8	
Jaybird	3.7	1.4	
Austin	1.9		
Andy	7.5		
Searle	1.7		
Clyde	9.5		
Arlesio	2.0	2.5	
Compute		0.3	
Darnome		0.3	
Arthur		7.7	
Willowland		14.6	
Cash		8.2	
Eclain		3.9	
Bellwod		7.4	
Abe		5.4	
Bemer		3.8	
Black Jack		9.2	4.0
Goliat			0.5
Sergio			22.1
Cococho			3.1
Gringo			7.1
Direction			15.4
Polo			43.3
Beton			4.5

Data was provided courtesy of CONAPROLE.

APPENDIX 2. Seasonal services per bull as % of total inseminations for two heifer rearing farms. Manuscript 1.

	1993				1994			
	WINTER		SPRING		WINTER		SPRING	
	Farm 4	Farm 3	Farm 4	Farm 3	Farm 4	Farm 3	Farm 4	Farm 3
David	3.5	5.0	73.3	48.2		5.1		49.7
Bovaliant	54.8			36.7				
Emperador	41.7	36.3				34.2		
Dylan					39.4		25.3	
Willow					28.0		6.6	
Windjammr					32.6		41.1	
Mountain Boy							27.0	
5720		27.3				22.7		
6032						0.9		
6037		31.4				37.1		
1690								20.9
2134								25.6
546								3.8
2531				15.1				
924			17.1					
Galstar			9.6					

Data was provided courtesy of CONAPROLE and CARECO.

APPENDIX 3. Chi square analysis on proportion of cycling heifers overall conception and pregnancy rates for seasonal performance. Manuscript 1.

Proportion of cycling Heifers

	Season	Observed	Expected
Cycling	Winter	1932	1982
Cycling	Spring	1490	1440
No cycling	Winter	221	171
No cycling	Spring	74	124

chi2	Probability
37.96	< 0.01

Overall conception Rate

	Season	Observed	Expected
Pregnant	Winter	1706	1732
Pregnant	Spring	1284	1258
No pregnant	Winter	447	421
No pregnant	Sprring	280	306

chi2	Probability
3.4	0.06

Overall pregnancy rate

	Season	Observed	Expected
Pregnant	Winter	1706	1688
Pregnant	Spring	1284	1301
No pregnant	Winter	226	244
No pregnant	Spring	206	188

chi2	Probability
4.7	0.03

APPENDIX 4. Chi square analysis on proportion of cycling Heifers, overall conception and pregnancy rates for yearly performance. Manuscript 1.

Proportion of cycling Heifers

	Year	Observed	Expected
Cycling	1993	1467	1518
Cycling	1994	1955	1904
No cycling	1993	182	131
No cycling	1994	113	164
		39.00	< 0.01
		Chi 2	Probability

Overall conception Rate

	Year	Observed	Expected
Pregnant	1993	1268	1241
Pregnant	1994	1722	1749
No pregnant	1993	199	226
No pregnant	1994	233	247
		2.1	0.15
		chi2	Probability

Overall pregnancy rate

Pregnant	Year	Observed	Expected
Pregnant	1993	1268	1236
	1994	1722	1664
No pregnant	1993	381	333
No pregnant	1994	346	404
		chi2	Probability
		23.7	< 0.01

APPENDIX 5. Nutrient composition of consumed pasture for winter and spring trials. Manuscript 2.

Parameter	Source	Df	Type III SS	F value	P > F
Dry matter					
	TRIAL	1	62.8	3.07	0.0883
	TREAT	2	46.35	1.13	0.3338
	TRIAL*TREAT	2	13.67	0.33	0.7185
	Error	36	737.40		
Crude Protein					
	TRIAL	1	5.57	0.25	0.6173
	TREAT	2	41.01	0.93	0.4017
	TRIAL*TREAT	2	1.41	1.41	0.9683
	Error	37	811.55		
Acid detergent fiber					
	TRIAL	1	384.26	1.89	0.1771
	TREAT	2	256.48	0.63	0.5374
	TRIAL*TREAT	2	279.85	0.69	0.5083
	Error	38	7719.31		
Neutral detergent fiber					
	TRIAL	1	7.67	0.03	0.8549
	TREAT	2	243.00	0.54	0.5885
	TRIAL*TREAT	2	869.86	1.92	0.1597
	Error	39	8816.92		
ASH					
	TRIAL	1	0.38	0.46	0.4999
	TREAT	2	9.54	5.76	0.0064
	TRIAL*TREAT	2	0.29	0.17	0.8404
	Error	39	32.27		
Degradable CP					
	TRIAL	1	0.0006	0.05	0.8297
	TREAT	2	0.051	1.95	0.1613
	TRIAL*TREAT	2	0.021	0.79	0.4640
	Error	28	0.37		
Water soluble carbohydrates					
	TRIAL	1	3.36	0.82	0.4014
	TREAT	2	0.64	0.08	0.9260
	TRIAL*TREAT	2	3.35	0.41	0.6829
	Error	6	24.73		

APPENDIX 5. Nutrient composition of consumed pasture for winter and spring trials. Manuscript 2.

Parameter	Source	Df	Type III SS	F value	P > F
Dry matter					
	TRIAL	1	62.8	3.07	0.0883
	TREAT	2	46.35	1.13	0.3338
	TRIAL*TREAT	2	13.67	0.33	0.7185
	Error	36	737.40		
Crude Protein					
	TRIAL	1	5.57	0.25	0.6173
	TREAT	2	41.01	0.93	0.4017
	TRIAL*TREAT	2	1.41	1.41	0.9683
	Error	37	811.55		
Acid detergent fiber					
	TRIAL	1	384.26	1.89	0.1771
	TREAT	2	256.48	0.63	0.5374
	TRIAL*TREAT	2	279.85	0.69	0.5083
	Error	38	7719.31		
Neutral detergent fiber					
	TRIAL	1	7.67	0.03	0.8549
	TREAT	2	243.00	0.54	0.5885
	TRIAL*TREAT	2	869.86	1.92	0.1597
	Error	39	8816.92		
ASH					
	TRIAL	1	0.38	0.46	0.4999
	TREAT	2	9.54	5.76	0.0064
	TRIAL*TREAT	2	0.29	0.17	0.8404
	Error	39	32.27		
Degradable CP					
	TRIAL	1	0.0006	0.05	0.8297
	TREAT	2	0.051	1.95	0.1613
	TRIAL*TREAT	2	0.021	0.79	0.4640
	Error	28	0.37		
Water soluble carbohydrates					
	TRIAL	1	3.36	0.82	0.4014
	TREAT	2	0.64	0.08	0.9260
	TRIAL*TREAT	2	3.35	0.41	0.6829
	Error	6	24.73		

APPENDIX 6. Nutrient composition of silage for winter and spring trials. Manuscript 2.

Parameter	Source	Df	Type III SS	F value	P > F
Dry matter					
	TRIAL	1	62.87	3.07	0.0883
	TREAT	2	46.35	1.13	0.3338
	TRIAL*TREAT	2	13.67	0.33	0.7185
	Error	36	737.40		
Crude protein					
	TRIAL	1	33.14	0.84	0.3665
	TREAT	2	127.58	1.61	0.2136
	TRIAL*TREAT	2	29.67	0.37	0.6905
	Error	38	1507.42		
Acid detergent fiber					
	TRIAL	1	384.26	1.89	0.1771
	TREAT	2	256.48	0.63	0.5374
	TRIAL*TREAT	2	279.85	0.69	0.5083
	Error	38	7719.31		
Neutral detergent fiber					
	TRIAL	1	7.67	0.03	0.8549
	TREAT	2	242.99	0.54	0.5885
	TRIAL*TREAT	2	869.86	1.92	0.1597
	Error	39	8816.92		
ASH					
	TRIAL	1	0.38	0.46	0.4999
	TREAT	2	9.54	5.76	0.0064
	TRIAL*TREAT	2	0.29	0.17	0.8404
	Error	39	32.27		
Water soluble carbohydrates					
	TRIAL	1	0.41	0.06	0.8335
	Error	2	14.54		
Degradable CP					
	TRIAL	1	0.0009	6.73	0.0140
	TREAT	2	4.52	16754.40	0.0001
	TRIAL*TREAT	2	0.0004	1.68	0.2012
	Error	33	0.004		

APPENDIX 7. Nutrient composition of diets for winter and spring trials Manuscript 2.

Parameter	Source	df	Type III SS	F value	P > F
Dry matter					
	TRIAL	1	62.87	3.07	0.0883
	TREAT	2	46.35	1.13	0.3338
	TRIAL*TREAT	2	13.67	0.33	0.7185
	Error	36	737.40		
Crude protein					
	TRIAL	1	19.05	1.05	0.2895
	TREAT	2	630.04	19.09	0.0001
	TRIAL*TREAT	2	32.33	0.98	0.3850
	Error	37	610.46		
Acid detergent fiber					
	TRIAL	1	93.48	0.65	0.4235
	TREAT	2	988.63	3.46	0.0416
	TRIAL*TREAT	2	55.19	0.19	0.8251
	Error	38	5427.21	142.82	
Neutral detergent fiber					
	TRIAL	1	173.70	1.21	0.2781
	TREAT	2	3368.23	11.73	0.0001
	TRIAL*TREAT	2	348.52	1.21	0.3080
	Error	39	5598.58		
ASH					
	TRIAL	1	0.34	0.54	0.4655
	TREAT	2	34.12	27.08	0.0001
	TRIAL*TREAT	2	0.58	0.46	0.6338
	Error	39	24.57		
Water soluble carbohydrates					
	TRIAL	1	1.18	0.49	0.5086
	TREAT	2	0.32	0.07	0.9350
	TRIAL*TREAT	2	0.73	0.15	0.8617
	Error	6	14.32		
Degradable CP					
	TRIAL	1	0.0007	0.08	0.7804
	TREAT	2	0.024	1.41	0.2600
	TRIAL*TREAT	2	0.017	1.00	0.3799

APPENDIX 8. Analysis of variance on initial age, initial weight, initial body

APPENDIX 9. Analysis of variance on initial age, initial weight, initial body condition score (BCS), average daily gain (ADG), change in body condition score serum urea nitrogen (SUN), and service interval (1^{st} -2^{nd}) for the spring grazing trial. Manuscript 2.

Parameter	Source	df	Type III SS	F value	P > F
Initial age	TREAT	2	0.97	0.81	0.45
	HERD	70	308.43	7.33	< 0.01
	Error	126	75.70		
Initial weight	TREAT	2	625.62	2.41	0.09
	HERD	71	75167.86	8.17	< 0.01
	Error	133	17230.54		
Initial BCS	TREAT	2	0.04	3.23	0.04
	HERD	71	0.40	0.92	0.64
	Error	135	0.82		
ADG	TREAT	2	4.02	27.73	< 0.01
	HERD	71	0.08	1.04	0.41
	Error	129	9.36		
Change in BCS	TREAT	2	0.04	3.23	0.04
	HERD	71	0.40	0.92	0.649
	Error	135	0.82		
SUN	TREAT	2	989.19	14.33	< 0.01
	HERD	68	2616.37	1.11	0.32
	Error	71	2450.33		
Service interval	TREAT	2	17.74	1.76	0.18
	Error	35	176.07		

TREAT = dietary treatment HERD = replicate

APPENDIX 10. Analysis of variance on initial age, initial weight, average daily gain (ADG) of cycling and non-cycling heifers for winter and spring grazing trials. Manuscript 2.

Parameter	Source	df	Type III SS	F value	P > F
Winter trial					
Initial weight	TREAT	2	210.11	0.12	0.89
	CYCLE	1	7055.98	7.82	< 0.01
	TREAT*CYCLE	2	2536.43	1.41	0.2471
	Error	256	230963.34		
ADG	TREAT	2	4.22	6.41	< 0.01
	CYCLE	1	2.87	8.73	< 0.01
	TREAT*CYCLE	2	0.63	0.96	0.39
	Error	251	82.57		
Spring trial					
Initial weight	TREAT	2	359.08	0.42	0.66
	CYCLE	1	2084.20	4.84	0.03
	TREAT*CYCLE	2	2653.06	3.08	0.05
	Error	201	86540.71		
ADG	TREAT	2	4.76	36.27	< 0.01
	CYCLE	1	1.64	24.99	< 0.01
	TREAT*CYCLE	2	0.32	2.41	0.09
	Error	197	12.94		

TREAT = dietary treatment CYCLE= cycling heifers

APPENDIX 11 Analysis of variance on weight at start of profiles, body condition score (BCS) at start of profiles, average daily gain (ADG), change in BCS and serum urea nitrogen (SUN) for a selected group of heifers for winter and spring grazing trials. Manuscript 2.

Parameter	Source	Df	Type III SS	F value	P > F
Winter trial					
ADG	TREAT	2	0.74	11.77	0.0002
	Error	26	0.82		
Change in	TREAT	2	0.04	3.89	0.0332
BCS	Error	26	0.12		
BCS start of	TREAT	2	0.049	0.13	0.8828
Profiles	Error	26	5.09		
Weight start	TREAT	2	5893.45	1.62	0.2179
of profiles	Error	26	47388.00		
Spring trial					
Change in	TREAT	2	0.01	1.13	0.3383
BCS	Error	26	0.17		
ADG	TREAT	2	0.21	3.39	0.0493
	Error	26	0.81		
BCS start of	TREAT	2	0.16	0.45	0.6442
Profiles	Error	25	4.34		
Weight start	TREAT	2	261.99	0.15	0.8645
of profiles	Error	25	22356.01		

TREAT = dietary treatment

APPENDIX 12. Analysis of variance on mean serum urea nitrogen (SUN), and progesterone at day 0, 2 and 14 of estrous cycle for winter trial and days 0, 3 and 14 for spring trial, for a selected group of heifers. Manuscript 2.

Parameter	Source	Df	Type III SS	F value	P > F
Mean SUN	TRIAL	1	1649.37	76.52	0.0001
	TREAT	2	2461.99	57.11	0.0001
	TRIAL*TREAT	2	72.50	1.68	0.1874
	Error	399	8600.22		
Winter trial					
Progesterone	TREAT	2	0.22	1.83	0.1844
Day 0	Error	22	1.33		
Progesterone	TREAT	2	0.69	3.86	0.0461
Day 2	Error	14	1.26		
Progesterone	TREAT	2	8.68	0.42	0.663
Day 14	Error	23	238.57		
Spring trial					
Progesterone	TREAT	2	0.03	0.50	0.6129
Day 0	Error	25	0.79		
Progesterone	TREAT	2	1.59	5.77	0.0084
Day 3	Error	26	3.58		
Progesterone	TREAT	2	12.49	2.39	0.1152
Day 14	Error	22	57.54		

TREAT = dietary treatment

APPENDIX 13. Chi square analysis on cycling for winter grazing trial. Manuscript 2.

Proportion of cycling heifers, % of all heifers

	Treatment	Observed	Expected
Cycling	A	78	74.71
Cycling	B	75	74.71
Cycling	C	72	75.57
No cycling	A	9	12.29
No cycling	B	12	12.29
No cycling	C	16	12.43

chi2	Probability
2.23	0.33

Proportion of cycling heifers within 6 days of PG treatment % of all heifers

	Treatment	Observed	Expected
Cycling	A	56	50.81
Cycling	B	44	50.81
Cycling	C	53	51.39
No cycling	A	31	36.19
No cycling	B	43	36.19
No cycling	C	35	36.61

chi2	Probability
3.59	0.17

Proportion of cycling heifers after 6 days of PG treatment % of all heifers.

	Treatment	Observed	Expected
Cycling	A	22	23.91
Cycling	B	31	23.91
Cycling	C	19	24.18
No cycling	A	65	63.09
No cycling	B	56	63.09
No cycling	C	69	63.82

chi2	Probability
4.64	0.10

APPENDIX 14. Chi square analysis on 1st service Conception rate for winter grazing trial. Manuscript 2.

Conception rate

	Treatment	Observed	Expected
Pregnant	A	59	59.97
Pregnant	B	55	57.67
Pregnant	C	59	55,36
No pregnant	A	19	18.03
No pregnant	B	20	17.33
No pregnant	C	13	16.64

chi2	Probability
1.64	0.44

Conception rate within 6 days PG treatment

	Treatment	Observed	Expected
Pregnant	A	44	42.09
Pregnant	B	30	33.07
Pregnant	C	41	39.83
No pregnant	A	12	13.91
No pregnant	B	14	10.93
No pregnant	C	12	13.16

chi2	probability
1.63	0.44

Conception rate after 6 days PG treatment

	Treatment	Observed	Expected
Pregnant	A	15	17.72
Pregnant	B	25	24.97
Pregnant	C	18	15.31
No pregnant	A	7	4.28
No pregnant	B	6	6.03
No pregnant	C	1	3.69

chi2	probability
4.59009	0.10

APPENDIX 15. Chi square analysis on 2nd service conception rate for winter grazing trial. Manuscript 2.

Conception rate

	Treatment	Observed	Expected
Pregnant	A	9	9.54
Pregnant	B	6	6.73
Pregnant	C	8	6.73
No pregnant	A	8	7.46
No pregnant	B	6	5.27
No pregnant	C	4	5.27

chi2	Probability
0.79	0.67

Conception rate within 6 days PG treatment.

	Treatment	Observed	Expected
Pregnant	A	3	5.00
Pregnant	B	5	5.00
Pregnant	C	7	5.00
No pregnant	A	8	6.00
No pregnant	B	6	6.00
No pregnant	C	4	6.00

chi2	Probability
2.93	0.23

APPENDIX 16. Chi square analysis on overall conception
In addition, pregnancy rates for winter grazing trial. Manuscript 2.

Overall Conception rate

	Treatment	Observed	Expected
Pregnant	A	68	65.08
Pregnant	B	61	65.08
Pregnant	C	67	65.83
No pregnant	A	19	21.92
No pregnant	B	26	21.92
No pregnant	C	21	22.17

chi2	Probability
1.62	0.44

Overall Pregnancy rate

	Treatment	Observed	Expected
Pregnant	A	68	67.95
Pregnant	B	61	65.33
Pregnant	C	67	62.72
No pregnant	A	10	10.05
No pregnant	B	14	9.67
No pregnant	C	5	9.28

chi2	Probability
4.50	0.11

APPENDIX 17. Chi square analysis on cycling for spring grazing trial. Manuscript 2.

Proportion of cycling heifers, % of all heifers

	Treatment	Observed	Expected
Cycling	A	57	50.60
Cycling	B	55	52.07
Cycling	C	42	51.33
No cycling	A	12	18.40
No cycling	B	16	18.93
No cycling	C	28	18.67

chi2	Probability
10.02	< 0.01

Proportion of cycling heifers within 6 days of PG treatments, % of all heifers.

	Treatment	Observed	Expected
Cycling	A	44	41.27
Cycling	B	43	42.46
Cycling	C	38	41.27
No cycling	A	25	27.73
No cycling	B	28	28.54
No cycling	C	31	27.73

chi2	Probability
1.11	0.57

Proportion of cycling heifers after 6 days of PG treatment, % of all heifers.

	Treatment	Observed	Expected
Cycling	A	13	9.57
Cycling	B	12	9.85
Cycling	C	4	9.57
No cycling	A	56	59.43
No cycling	B	59	61.15
No cycling	C	65	59.43

chi2	Probability
5.73	0.06

APPENDIX 18. Chi square analysis on 1st service conception rate for spring grazing trial. Manuscript 2.

Conception rate

	Treatment	Observed	Expected
Pregnant	A	42	37.38
Pregnant	B	38	36.07
Pregnant	C	21	27.55
No pregnant	A	15	19.62
No pregnant	B	17	18.93
No pregnant	C	21	14.45

chi2	Probability
6.48	0.039

Conception rate within 6 days PG treatment.

	Treatment	Observed	Expected
Pregnant	A	30	27.81
Pregnant	B	29	27.18
Pregnant	C	20	24.02
No pregnant	A	14	16.19
No pregnant	B	14	15.82
No pregnant	C	18	13.98

chi2	Probability
2.63	0.27

Conception rate after 6 days PG treatment

	Treatment	Observed	Expected
Pregnant	A	12	9.86
Pregnant	B	9	9.10
Pregnant	C	1	3.03
No pregnant	A	1	3.14
No pregnant	B	3	2.90
No pregnant	C	3	0.97

chi2	probability
7.58	0.02

APPENDIX 19 Chi square analysis on 2nd service conception Rate for spring grazing trial. Manuscript 2.

Conception rate

	Treatment	Observed	Expected
Pregnant	A	7	8.49
Pregnant	B	10	9.62
Pregnant	C	13	11.89
No pregnant	A	8	6.50
No pregnant	B	7	7.38
No pregnant	C	8	9.11
No pregnant			

chi2	Probability
0.88	0.64

Conception rate of heifers bred within 6 days PG treatment.

	Treatment	Observed	Expected
Pregnant	A	7	7.9
Pregnant	B	8	7.9
Pregnant	C	11	10.17
No pregnant	A	6	6.09
No pregnant	B	7	6.09
No pregnant	C	4	7.83

chi2	Probability
0.40	0.82

APPENDIX 20 Chi square analysis on overall conception
In addition, pregnancy rates for spring grazing trial. Manuscript 2.

Overall conception rate

	Treatment	Observed	Expected
Pregnant	A	49	48.48
Pregnant	B	48	46.79
Pregnant	C	34	35.73
No pregnant	A	8	8.51
No pregnant	B	7	8.21
No pregnant	C	8	6.27

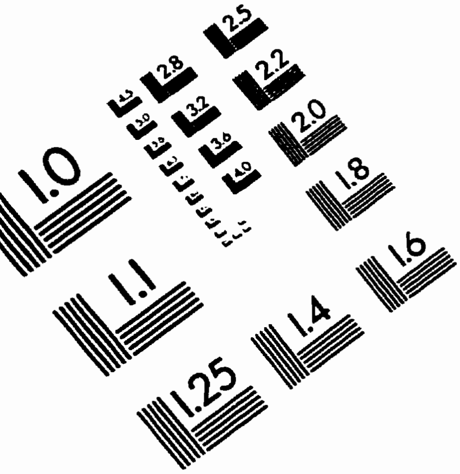
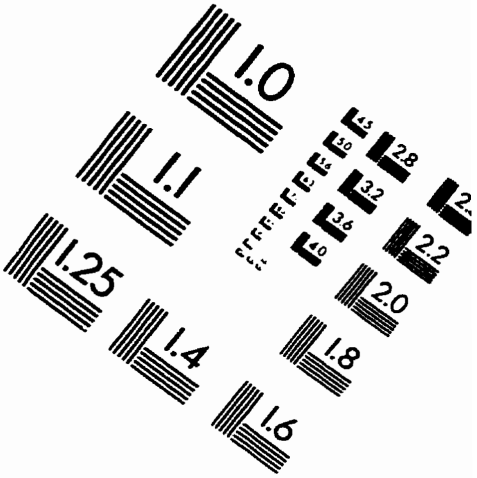
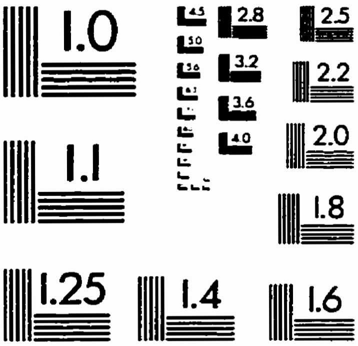
chi2	Probability
0.81	0.67

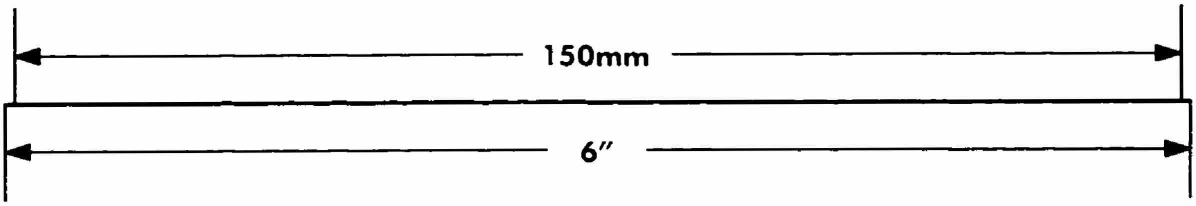
Overall pregnancy rate

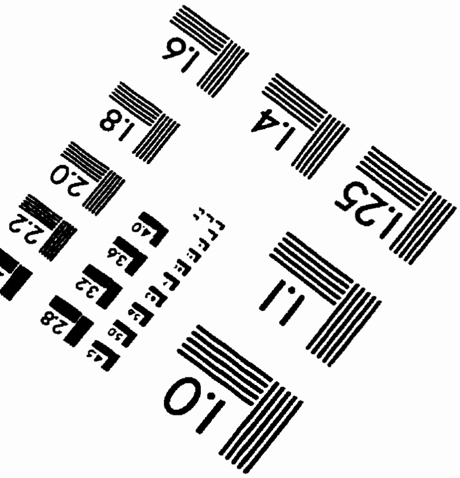
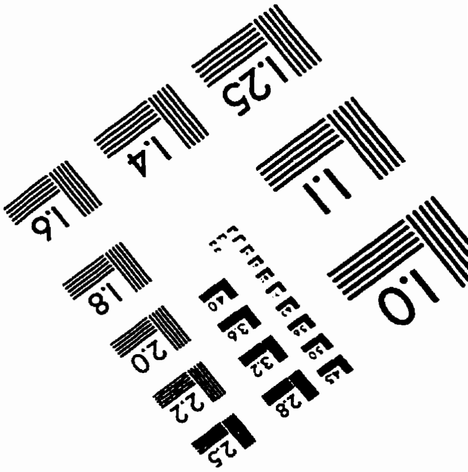
	Treatment	Observed	Expected
Pregnant	A	49	43.25
Pregnant	B	48	44.50
Pregnant	C	34	43.25
No pregnant	A	20	25.75
No pregnant	B	23	26.50
No pregnant	C	35	25.75

chi2	Probability
8.09	0.02

IMAGE EVALUATION
TEST TARGET (QA-3)



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