FEEDING STRATEGIES AND NUTRIENT MANAGEMENT OF GRAZING CATTLE IN URUGUAY

By

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CHAPTER I

INTRODUCTION

World population has been growing at substantial rates during this last century increasing the demand for food. This increment in food demand has spur agricultural food production by doubling it in the last 35 years but associated with an increment of 6.87 fold increase in nitrogen fertilization, a 3.48 fold increase in phosphorus fertilization, a 1.68 fold increase in cropland irrigated and a 1.1 fold increase in land cultivation (Tilman, 1999). At the same time, there has been an increasing concern of the public for environmental and water quality issues that has led to more regulations, codes or laws. Each of these regulations that affect cattle producers have resulted in alterations in management practices (Morse, 1996).

It is clear that sustainable practices are needed in order to increase food production without detrimental consequences for the environment. The need for studies that involve agrosystems can give us a clue for pointing out more efficient and environmental friendly production systems. Since nutrient management involves the integration of several aspects of farm agrosystems it has been visualized as an important tool to identify not only the more efficient management practices but also the type of practices that contribute to non-point pollution.

One of the key factors that can reduce the environmental impact from animal operations is to optimize the level at which nutrients are added to diets and minimize excesses. Feeding strategies that can reduce labor and nutrient loads to the environment can have an important impact. With the help of more accurate models to predict

requirements, producers can maintain performance while reducing environmental impacts (NRC, 1996). One model when applied in a dairy reduced nitrogen excretion by 25% while also reducing feed costs (Fox et al., 1995).

A challenge for animal nutritionists is to curtail adverse environmental impact by reducing nutrient losses and increasing nutrient recovery in edible products from the animal, while maintaining or enhancing productivity and/or economical benefits.

The objective of this research was 1) to quantify mass nutrient balances of five dairy agrosystems of Uruguay, and 2) to test the impact of the feeding frequency of cracked corn on intake, digestibility, ruminal parameters, average daily gain and as a tool for decreasing labor.

CHAPTER II

REVIEW OF LITERATURE

In the last decades farms have been going through structural and technological changes. There has been an increase in the number of animals per farm, specialization of the farms and a larger number of confined animals. These factors have increased the amount of manure produced within the farm and the potential of polluting the environment. An increase in public concern for the environment has resulted in greater pressure for regulating manure management and minimizing possible contamination resulting in a series of changes in production practices. During the last 35 years agricultural food production has been doubled but it was associated with a 6.87 fold increase in nitrogen fertilizer utilization and a 3.48 fold increase phosphorus fertilizer utilization (Tilman, 1999).

In addition nutritionists usually recommend higher mineral concentrations relative to requirements due, among various possible reasons, to the fact that they feel NRC recommendations are not meet under practical conditions, that some minerals requirements are poorly described, and to provide a safety margin to prevent any likelihood of deficiencies (Spears, 1996). In diets analyzed by North Carolina feed testing laboratory the median concentration used in dairy diets for many minerals were several times higher (calcium 1.77; phosphorus 1.32; sodium 1.78; iron 9.76; zinc 2.70 for the ratio median:requirement) than the NRC requirements for lactating dairy cows and even

greater values for pigs (Spears, 1996). All these factors have increased pollution potential from farms into the environment.

Nutrient management has been seen as a possible tool for balancing the amounts of nutrients within a farm and reducing the possibility of polluting the environment. In this review, nutrient management, metabolism of nitrogen, phosphorus and potassium in the ruminant and effects of some feeding and supplementation strategies will be discussed.

1. What is nutrient management?

Nutrient management involves the integration of several aspects of the farm operation (Klausner, 1995b). Nutrient management is a mass balance that begins with an accurate quantification of nutrients entering the livestock production system and nutrients leaving the system (Tyrell, 2001). Within the farm boundary there are inputs of nutrients (feed, fertilizer, legume N and rainfall), outputs (animal products and crops) and losses to the environment (ammonia volatilization, leaching, denitrification, runoff, and erosion) (Klausner, 1995a).

Farm fields are potentially non-point sources of pollution in which discharges of nitrates by leaching through the plant root zone, actually may be invisible to the normal observer (Lanyon, 1994). Nutrient management is the internationally accepted strategy for addressing non-point farm pollution (Beegle et al., 2000). The consequences of non point pollution may be seen far from the original discharged area, and the benefit of reducing this type of pollution may only partially accrue to the polluter (Lanyon, 1994). Also the problems with nutrient pollution are not generally the result of mismanagement

by farmers but the result of evolving agricultural systems with no direct costs associated with environmental quality (Beegle et al., 2000).

Nutrient management implies different components in the farm daily activities such as feed, manure management (collection, storage and application), fertilizer and crop selection, close records of the nutrients and the use of each paddock on the farm. This will be discussed in more detail within each system (dairy or beef).

The nutrients most implicated in pollution of the environment are nitrogen (N) and phosphorus (P) (Pell, 1992). In farms where excessive manure is used potassium (K) can be up to 4% of the haylage and can create nutritional problems when balancing a dairy ration for transition cows (Pell, 1992).

1.1. Nitrogen, Phosphorus and Potassium flux and cycle within a farm.

There are several general reviews for nitrogen (Cowling and Galloway, 2001; Galloway, 1998; Lin et al., 2000; van der Hoek, 1998) and phosphorus (Higgs et al., 2000; Smil, 2000) cycles that the reader can referred if interested in these subjects. This section will refer to the flux of these nutrients (N, P) and K within the farm boundaries.

Although all nutrient cycles are complex, the N cycle is particularly complex because N suffers changes in valency and state (usually mitigated by microbial transformation and action) and can form water soluble and gaseous compounds with considerable potential to escape from agricultural control (Jarvis et al., 1995). Nitrogen may enter the farm mainly as purchased feed, animals, and fertilizer or by legume fixation. The big concern with N is its high potential to move through soils and possible contamination of water threatening human and animal health from excess of nitrates

(NO_3) in water. Infants are sensitive to NO_3 that may cause blue baby syndrome (Hart et al., 1997).

There are several processes regarding N in the soil being the majors, mineralization, nitrification, denitrification, immobilization and ammonia volatilization (Klausner, 1995b). During mineralization organic nitrogen decomposes first to ammonia and afterwards ammonium is produced. In the process of nitrification ammonium is converted into nitrite and afterwards into nitrate being more rapidly if soil conditions are warm, moist and well aerated. During denitrification nitrate is converted into nitrite, which can be converted either to nitric oxide or through intermediates to nitrous oxide and released to the atmosphere. This last process occurs more rapidly if the soil is poorly aerated and or waterlogged. In the process of immobilization available, N in the soil is bound to the microbial biomass. Volatilization of ammonia can occur also when urea fertilizers or manure is applied onto the soil surface and not incorporated. Gaseous N losses, either through ammonia volatilization or through denitrification losses (N_2 and N oxides) are very difficult to quantify because of their dependence on environmental conditions and microbial activity (Sharpley et al., 1998).

Nitrogen in plants is absorbed mainly in two forms as nitrate or as ammonium. Nitrate, an anion, is not absorbed by the clay or organic matter in the soil, and is readily absorbed by plants or susceptible to leaching or denitrification since it is in the soil solution (Whitehead, 1995). Ammonium is a cation, is retained by cation exchange on the clay and organic matter and is less accessible to roots and to potentially pollute groundwater (Whitehead, 1995). The physiological control over the absorption of N may be disturbed by defoliation (grazing, cutting, harvesting) and return to previous

absorption rates does not occur for several days after defoliation (Jarvis and Macduff, 1989). Nitrogen is under many circumstances the second limiting nutrient to grass sward potential while water is first limiting (Jarvis et al., 1995). Under conditions of no limiting nutrient, herbage yield is controlled by the amount and timing of nitrogen fertilizer applications (Hemingway, 1999). Grass fertilization with N has different effects while dry matter production per unit of surface is increased in a curvilinear manner, crude protein in increased linearly (Kemp et al., 1979). The fertilization policy and the type of forage are also important in the losses of nitrogen to the environment. A study comparing the ratio of NH_3 losses in swards grazed by cattle one from ryegrass fertilized with 420 kg fertilizer N/ha and the other from a mixed ryegrass/white clover sward with no N fertilizer was 7:1 (Ryden et al., 1987). Results from several investigations suggest that fertilizer N, even when applied to grassland regularly has no effect on soil N unless the soil is extremely low in organic matter (Whitehead, 1995).

In productive grass clover swards legumes may be able to fix between 100-300 kg of N ha^{-1} $year^{-1}$ depending in the vigor of the clover (Whitehead, 2000). In ungrazed grasslands up to 100 kg N ha^{-1} $year^{-1}$ can be transferred to grasses meanwhile in heavily grazed the same amount can be added by the excreta (Whitehead, 2000).

Forage quality can be manipulated by management, time of harvest and fertilization as the more important factors (Tamminga, 1996). Quality usually associated with higher digestibility is very important in order to reduce animal excretion of nutrients since a higher digestibility is synonym for fewer nutrients excreted.

The two primary forms of N in manure are ammonia and organic N being urine the major source of ammonia (Van Horn et al., 1994). About 50 % of the N in fresh

manure is present as urea that converts rapidly into ammonia and may be lost rapidly through volatilization (Klausner, 1995a). Urine is the major excretion pathway for rapidly available fertilizer-N (Powers and Van Horn, 2001). In an experiment in which ryegrass swards received either 420 or 210 kg N/ha per year where compared to a mixed ryegrass/white clover sward receiving no mineral N there were little differences in fecal N but there were differences in urine N of Friesian steers (74, 60 and 56 % of excreted returns, respectively) (Jarvis et al., 1989). The ammonia losses were equivalent to the urine losses and these authors found that there were little differences between the effects with the 210 N treatments and the grass/clover treatment. High N fertilization may reduce N efficiency by animals. The actual efficiency of N utilization by dairy cows in intensively managed pastures with high N fertilization is 16% when theoretically a 600 kg cow producing 25 kg of milk can have a maximum efficiency of 40-45 % (Van Vuuren and Meijs, 1987). On the other hand a decrease in N fertilization decreased organic matter digestibility (.02 units) but did not affect the site of digestion when comparing grass species harvested at the same age of regrowth (Peyraud and Astigarraga, 1998). Also dry matter production can be decreased with lower N fertilization having the farmer to reduce stocking rate and perhaps reducing its total farm income.

Phosphorus is less mobile in the soil than N. Phosphorus in water is not considered to be toxic to animals and humans. However, it has become of greater concern because of the possibility of producing eutrophication in surface waters (Sharpley et al., 1998). Even small amounts of P can increase the concentration above the critical value of eutrophication (Higgs et al., 2000). Although carbon (C) and N are with P the three elements to be concerned in eutrophication, fresh air water exchange, fixation of nitrogen

by blue green algae and photosynthesis make P the limiting element (Sharpley et al., 1998) and responsible of triggering the cycling of C and N (Smil, 2000). Aquatic nutrient eutrophication can lead to the loss of biodiversity, outbreak of nuisances' species and impaired of fisheries among others (Tilman, 1999).

Although variations between organic and inorganic forms of P in the soil can range from 10 to 90 % in most of the soils, including grasslands more than 50 % is inorganic (Whitehead, 2000). The inorganic forms of phosphate in the soil can be (i.) Phosphate present in the soil solution ($H_2PO_4^-$ and HPO_4^{2-}) (ii.) insoluble calcium phosphates (iii.) Phosphate adsorbed and possibly occluded by hydrous oxides (iv.) Phosphate adsorbed by clays, and (v.) phosphate in various unweathered minerals (Whitehead, 2000).

There are three main routes by which P can be lost from land these are in eroded soil, by surface runoff and leachate (Higgs et al., 2000). The adsorption of P by soil material usually increases the probability of surface runoff rather than leaching (Sharpley et al., 1998). However, the P adsorption capacity of soils is not unlimited and can leach if it is present in high concentrations in soils.

Plant take P as either $H_2PO_4^-$ or HPO_4^{2-} and the relative form depends of the pH usually the former is in higher concentration but as soil pH increases the proportion of the latter increases (Whitehead, 2000). According to this author the rate of absorption depends partly on the concentration in the soil solution near the root surface and partly by the rate of movement of the ions towards root surface.

A significant amount of P (almost 85%) in forages is present as phytic acid that in ruminants is completed hydrolyzed having a true availability of .65 to 1.0 (Minson,

1990). Usually about 95 % of the P is excreted in the feces (Tamminga, 1992). An increase in the urine excretion occurs when the concentration in the plasma increases exceeds the renal threshold of 60 to 90 mg P/L (Challa et al., 1989). At dietary concentrations of 0.24 % P, almost 75 % of P in feces is in the form of inorganic P (Barrow, 1987).

During the storage of slurry some mineralization of organic P occurs increases the amount of the inorganic form that can reach 80 % of total P (Whitehead, 2000). Plants equally absorb inorganic P from slurry as inorganic P fertilizer (Tamminga, 1992).

Potassium is more mobile than P in soils, however, it does not represent a threat to ground and surface water (Hart et al., 1997; Klausner, 1995b) or at least that we are aware (Paul, 1999). There is a maximum value for drinking water of 12 mg K/L but harmful effects are not well-documented and other foods such as milk may have values up to 1,500 mg K/L (Tamminga, 1996).

Most of the soils have an abundant supply of K, but only a very small part of it is readily available to plants (Cherney et al., 1998). Soil K is in various forms such as (i.) K in soil solution (ii.) ready exchangeable K (iii.) K changeable with difficult and (iv.) mineral K (Cherney et al., 1998). Clay soils have greater potassium firstly due to negative charge of the clay (Paul, 1999) and secondly because it is present in igneous rocks such as biotite micas (Whitehead, 1995).

Forage grasses are luxury consumers of potassium, the greater the availability the greater the K concentration (Cherney et al., 1998; Paul, 1999). Absorption of K depends on its concentration in the soil solution near the root zone (Whitehead, 2000). If this concentration is low (less 1mM), uptake across the plasma membrane is thought to be

mediated by a carrier protein across the plasma membrane releasing the K inside the cell but at higher K concentrations is transported increasingly by a relatively passive mechanism (Whitehead, 2000). The content of K and P in forage depends on herbage maturity within the growth cycle and the content of both decreases with declining crude protein concentrations (Hemingway, 1999). In an experiment carried out in New Zealand (Carran and Theobald, 2000) were they compared a system that had been 23 years with and without excreta, exchangeable K (available) was higher for the system with excreta than the one without.

If potassium levels in forages increase and exceed 3 % it may bring problems in dry cow nutrition such as milk fever, hypocalcemia, downer cow syndrome and in severe cases death (Hart et al., 1997; Horst and Goff, 1997).

Potassium in the diet of the animal is usually returned mainly through urine. Potassium in manure is mainly in a soluble form that is almost all readily available to plants and can be substituted for fertilizer K in a one to one basis (Klausner, 1995b).

1.2. Sustainability

Sustainability definitions have been address in many reviews and related to many different topics related to agriculture (George, 1999; Gibon et al., 1999; Hansen, 1996; Mebratu, 1998).

The World Conservation Union, United Nations Environment Program, and the World Wide Fund defined sustainable development for Nature in their report caring for nature (IUCN/UNEP/WWF, 1991) as " improving the quality of human life while living within the carrying capacity of supporting ecosystems".

Sustainability may become the first limiting factor according to the new USDA-EPA requirements for comprehensive nutrient management planning to protect water quality mainly in dairy farms (Fox et al., 2000).

It is important to start taking into account this issue in all the agricultural systems and within the definition above stated nutrient management appears as an important tool to achieve it.

1.3. Nutrient Management in Dairy Farms

Conditions change through different countries and states, however, there are several concepts that can be applied for every situation in what nutrient management concerns and really what changes are the parameters to be used in each particular situation (Bouldin and Klausner, 1998).

One of the more important components of nutrient management is how much feed and fertilizer are introduced to the farm and this is a function of the herd requirement, production targeted (crops and animals), and diet balance. Dairy production in most parts of the world has become strongly dependent of inputs to the farm mainly fertilizer and purchased feeds adding nutrients (Goh and Williams, 1999). In grassland systems that include legumes, fixation of N is an important source of input of N, most of the time as the most important as in many farms in New Zealand, Argentina, and Uruguay.

Nutrition has a big impact in the amount of nutrients we will have latter to dispose. Several reviews have addressed this topic (Powers and Van Horn, 2001; Tamminga, 1992; Tamminga, 1996; Tamminga and Verstegen, 1996; Van Horn et al.,

1996). In general the more balanced are requirements and feed and the higher its digestibility the lower the load of nutrients and the volume of the manure.

Nutrient management includes an important component that is called best management practices (BMP) of animal wastes. Best management practices are proper procedures and farming methods that look after a correct utilization of manure for an optimal plant growth and to minimize problems to the environment (Hammond et al., 1994; Lilly, 1991; Moore Jr, 1998). BMP's practices include proper nutrient management using agronomic rates of N and/or P (Eghball and Power, 1994; Klausner, 1995b; Moore Jr et al., 1995). Since N:P ideal relation for most of the crops is 8:1 (Moore Jr et al., 1995), generally we are adding more P than needed and soil accumulation will occur (Moore Jr et al., 1995; Van Horn et al., 1996). This occurs because manures are richer in P relative to N due to volatilized NH_3, denitrification under wet conditions and ability of many crops to luxury consume N (Van Horn et al., 1996).

Kohn et al (1997) made a sensitivity analysis of nitrogen losses from dairy farms and they concluded that improvements in animal diets would increase total farm N efficiency by 48 % through herd nutrition and crop management while targeting only manure management would be about one fourth as effective.

Due to the difficulty in measuring N losses to the environment in farms usually simulations of different scenarios are run through Monte Carlo techniques. St Pierre and Thraen (1999) simulated animal grouping strategies and economic factors and found that a 25 % increase in milk production reduced N excretion per kilogram of milk produced by 8%. Furthermore, understanding and controlling the effect of feed composition in the metabolism of the animal may reduce by an additional 8 % of the N excreted. Using the

same techniques Velthof and Oenema (1997) compared three farms with different nutrient management. The farm that included not only manure management but also in addition N nutrition care in the diet of cattle showed the lower emission of nitrous oxide (N_2O), less than half that the farm that included only manure management.

Validating a N planner in two farms, Dou et al., (1998) found that a good overall management practice integration for one of the farms could not be enough without an animal ration balancing. The other farm in the study not only needed to improve feeding strategy but also crop selection and manure management. It is clear that when trying to minimize N pollution without affecting crop and animal production all components of a farm system should be considered.

Klausner (1995a) formulated a nutrient mass balance for N in several New York dairies of different sizes (45-1300 cows) and found the percentage N remaining on the farm expressed as N % varied from 61 to 76 percent.

Most of the N that is introduced in the farm stays on it or it is lost to the environment but it is not incorporated into animal or crop products (Aarts et al., 1992; Aarts et al., 2000a). Dairy cows on average use 25 to 30 % of the N that they consume (Chandler, 1996). As milk production increases, the excretion of N in urine and feces decreases per unit of milk produced (Chandler, 1996; St-Pierre and Thraen, 1999).

The percentage of protein in the diet has been studied by several authors (Cunningham et al., 1996) (Komaragiri and Erdman, 1997; Wu and Satter, 2000b) from a point of view of reducing nitrogen excretion in diets with corn silage, alfalfa and corn grain. Increments over 17 % of crude protein show little increment in milk production. Analyzing several experiments, Satter et al (2001) concluded that in a practical approach

is it possible to reduce from 17.5-18.5% to 16% through more precise ration balancing in high producing cows with the result of a 10-15% reduction in dietary N and 13 to 20% reduction in excreted N.

Dietary N usually not only affects N excreted but also affects relative amounts in urine and feces as was discussed in a previous section. Usually a decrease in total N in the diet decreases N in the urine, which is more unstable than the N excreted in feces.

Satter et al., (2001) suggest three strategies to reduce nitrogen excretion and these are an increase in the amount of microbial protein synthesized in the rumen, a balance between rumen undegradable protein (RUP) and rumen degradable protein (RDP), and balance of the amino acids mainly the essentials in the feed.

For the case of phosphorus we can control the entrance of it to the farm, usually may come in the feed or fertilizer, and under most of farm conditions is less probable to be lost to the environment. Usually the big concern is the buildup of P in soils heavily manured or that have not been used taking into account the requirements of crop and pastures producing a nutrient imbalance in the farm. Low mobility phosphorus also makes P depletion a gradual process being underestimated the cost of nutrient depletion and the benefits of fertilization with one season experiments (van Noordwijk, 1999).

In the Netherlands, dairy farms occupy 64 % of the land, it is calculated that 67 % of purchased P in feed or fertilizer doesn't leave the farm in the form of milk or meat (Aarts et al., 2000b). In this country farmers need to take close notes of inputs and outputs of nutrients (bookkeeping) with special emphasis to P and N in manure (Breembroek et al., 1996).

The possibility of reducing the amount of P in the feed to meet requirements and better crop-pasture management are the keys for a successful reduction of P to be loss to the environment. Reducing P in the diet by 33 % (.32% vs. .48%) resulted in P concentrations of feces and runoff from manure application of 52% and 10 times less (Satter et al., 2001).

Dairy cows usually excrete less than 1g P/d in urine but when fed 20 to 30 % more P than requirements they may excrete 3-5 g of P/d (Wu and Satter, 2000a). So overfeeding P may convert excreted P in more soluble compounds, which latter can be more suitable to be lost to the environment. According to Satter et al (2001) in U.S. dairy it is possible to reduce dietary P from .45-.50 to .36-.40%, which represents a 20% reduction in dietary P.

Usually in systems that rely more on concentrates the most important of P input is the feed (concentrate) while systems in New Zealand where pastures are a higher percentage of the diet, fertilizer is the main P input (Goh and Williams, 1999).

1.4. Nutrient Management in Beef Systems (range and feedlot)

In extensive rangelands cattle may usually graze underdeveloped and unfertilized swards. Usually the level of nutrient extraction is low (3 kg N/ha) as the annual inputs mainly coming from atmospheric deposition (2-10kg N/ha); (Goh and Williams, 1999). Losses by volatilization are relatively high but still low when compared with more intensive beef systems and may be affected by the presence or not of dung beetles. Usually the greater concern in these systems is the possibility of overgrazing which may lead to soil erosion and surface water contamination (Goh and Williams, 1999).

In improved grasslands the level of intensity increases, and there are more inputs such as fertilizer and fixation of N by legumes. While in extensive rangelands grass utilization may be 30 % (Coleman et al., 1977). In improved pasture systems utilization can reach 80 % and a cycling of almost 400 kg N ha^{-1} (Goh and Williams, 1999). If the improved grasslands include legumes usually for a high dry matter yield, high levels of P in the soil are required (20 ppm) although the amount will depend of the type of legume. There is not much information published on nutrient management in this type of system. Goh and Williams (1994) summarize 4 systems from the literature, rangeland, unfertilized ryegrass pasture, ryegrass white clover and fertilized rye grass pasture being their inputs of 9, 25, 175 and 435; outputs of 10, 19, 60 and 309 which imply total budget –1, 6, 115 and 126 (kg N ha^{-1}) respectively.

More concerns and more research have been done for feedlots due to the high level of intensification. In the U.S. 33 % of the beef production comes from feedlot (Eghball and Power, 1994).

Galyean (1999) reviewed aspects of nutrition either levels or forms of feeding with special concern to the environment. Feeding programs such as restricted feeding and phase feeding may decrease manure loads and the ability to accurately forecast nutrient input:output when the feed intake is fix (Galyean, 1999). In a survey of consultant nutritionists, reported by Galyean (1996), there was an increase of crude protein levels used by the consultants with respect to the factorial system of the 1984 NRC for beef cattle. However, this does not necessarily mean more nitrogen should be excreted. According to Galyean (2000), in the cases were dry matter intake (DMI) is less with a deficiency in degradable intake protein (DIP) an increase of DIP will increase DMI and

energy yield from the rumen will increase, increasing average daily gain (ADG) and increasing N retention. Therefore, as for dairy it is not only the levels of a nutrient that are given but also how balance with respect to other nutrients needed for efficient feedlot operations.

Analyzing 33 livestock operations in Nebraska (pigs and feedlots), Koelsch and Lesoing (1999) found that most of the farms had a substantial greater N inputs that managed outputs. Although there was great variability among farms these authors observed that formulation of diets and exporting manure nutrients to off-farm users had a great impact on reducing the nutrient imbalance.

Through nutrition it is possible to shift nitrogen losses to urine or feces. This may have a great impact on the amount of N that is volatilized from the pen. Using corn byproducts may shift fecal N to 50 % in urine and 50 % in feces (Bierman et al., 1999) while typical grain diets (85% grain) N in urine may account for 75 % (Satter et al., 2001). Erickson et al. (2000) through phase feeding were able to reduce N excretion and reduce volatilization.

Diet pH of urine and feces may affect N volatilization (Tucker and Watts, 1993). Lower pH enhances less volatilization since ammonia stays as ammonium.

Erickson et al. (1999) worked with yearling steers and observed that P requirements were not more than 0.14% of diet DM. The implications of this data is that P requirements for these animals seem to be below what is supplied by the grains in the diet (Satter et al., 2001). However, calves may need some P supplementation. Few experiments are reported in the literature with respect P requirements in the feedlots and further research is needed.

Manure and wastewater management in feedlots is important in order to control air (dust an odor), water (surface and ground) pollution, to improve environmental conditions, to recover nutrients and to comply with legislation (Sweeten, 1998).

2. Nitrogen Physiology of the Ruminant

The normal sources of nitrogen (N) for ruminants are dietary protein and non-protein nitrogen (NPN). These dietary sources include a large variety of nitrogen forms such as nucleic acids, amino acids, proteins, peptides, nitrates, nitrites, urea, ammonia, amines and amides (Huntington and Archibeque, 1999). The fate of these nitrogen sources in the animal will be discussed in the following sections.

2.1. Nitrogen metabolism in the rumen environment

The first step in nitrogen metabolism in ruminants usually occurs in the rumen. Bacteria are the principal rumen microorganism that are involved in protein metabolism (Broderick et al., 1991) while protozoa, and anaerobic fungus also carry out proteolysis, peptidolysis and deamination but to a lesser extent. More than 99 % of the rumen bacteria are strict anaerobes (Hungate, 1966). The microbial population of the rumen has a moderate proteolytic activity when compared with other proteolytic microbes (Wallace, 1996), but the length of time that particles are retained in the rumen allow a substantial breakdown of the dietary proteins (Broderick et al., 1991).

2.1.1. Protein, peptides and amino acids catabolism

Many of the predominant bacteria are proteolytic, but none of them are dependent on protein as its only energy source (Yokohama and Johnson, 1993). However, under most production conditions, the rumen bacteria are under energy limiting environments and may use the amino acids products of protein breakdown for energetic purposes (Wallace, 1994). Bacteria are the most important organism in breakdown of soluble protein in the rumen (Kopecny and Wallace, 1982). The principal proteolytic bacteria are *Bacteroides amylophilus, Prevotella ruminicola, Butyrivibrio fibrisolvens* and *Streptococcus bovis* (Russell et al., 1981; Wallace and Cotta, 1997). These bacteria act on the proteins through proteases that are mainly associated with the wall of the bacteria (Kopecny and Wallace, 1982). Protein breakdown begins either by adsorption of soluble protein to the bacterial surface, by adsorption of bacteria to insoluble protein (Wallace, 1985; Wallace, 1994), or by the ingestion of a particulate substrate by protozoa (Wallace, 1994). The final products of proteolysis are usually polypeptides, oligopeptides, dipeptides, amino acids and ammonia (Broderick et al., 1991).

Although microbial species are very dependent of the type of diet (Hazlewood et al., 1983), different animals under the same diet and housed together may have completely different patterns of proteolytic enzymes (Wallace and Cotta, 1997) adding therefore an individual differentiation. Oligopeptides are degraded mainly by the peptidase activity of bacteria (Wallace, 1994) while protozoal peptidases are more active over dipeptides (Wallace et al., 1990).

Peptides breakdown differ according to the type of peptide. The structure of the N-terminus is crucial in determining degradation (Wallace, 1997). If this N-terminus is

mainly near glycine or proline or if the peptide has a negative charge it will be slowly degraded (Wallace, 1997). The predominant mechanism of peptide hydrolysis by the rumen microorganisms is dipeptidyl peptidase (Wallace, 1996) being *Prevotella ruminicola* the most important. These bacteria may account for more than 60 % of the total flora of sheep, eating silage (Van Gylswyk, 1990) while *Selenomonas ruminatium* can constitute 22-51% of the ruminal bacteria of animals fed cereal grains (Stewart and Bryant, 1997).

Most of the proteolytic microbes are capable of carrying out deamination (Morrison and Mackie, 1996). The ammonia production from deamination of amino acids is carried out mainly by *Megasphaera elsdenii, Selomonas ruminatium* and a few *Butyrivibrio spp* (Yokohama and Johnson, 1993). Amino acids are the most important source of ammonia in the rumen (Chalupa, 1976). Proteolytic activity increases in the rumen by increasing diet fermentability probably due to increased microbial biomass in the rumen (Broderick et al., 1991). Wallace (1996) distinguished two types of ammonia producing bacteria the high number low activity *(Butyrivibrio fibriosolvens, Megasphaera elsdenii, P. ruminicola, Selenomonas ruminatium* and *Streptococcus bovis*) that are monensin resistant with an activity of 10-20 nmol NH_3 min^{-1} (mg protein)$^{-1}$ and the low numbers with high activity bacteria (*Clostridium aminophilum, Clostridium sticklandii,* and *Peptostreptococcus anareobius*) that are monensin sensitive with an activity of 300 nmol NH_3 min^{-1} (mg protein)$^{-1}$. Although is of greater importance the high number with low activity bacteria, both type of populations can have a major impact on the nitrogen (N) retained by the animal.

The ruminal protozoal population is composed of flagellates and ciliates being the latter the more numerous (Jouany, 1996). The total mass of protozoa in the rumen is almost the same as that of bacteria being 2% of the weight of rumen contents (Yokohama and Johnson, 1993). Protozoa uses bacterial and feed protein that are available in the rumen, according to Jouany et al (1988) this is the reason why feed protein degraded in the rumen generally decreases when animals are defaunated. The engulfment of bacteria by protozoa may be selective or nonselective depending of protozoa and bacteria (Coleman, 1986; Jouany et al., 1988; Jouany and Martin, 1997). Unlike bacteria, protozoa have no ureases and cannot use urea or ammonia to synthesize amino acids (Onodera et al., 1977). Protozoa have a greater ability to ingest particulate matter and therefore being more active in degrading insoluble rather than soluble protein (Jouany, 1996). Protozoal consumption of bacteria probably accounts for a significant part of protein turnover and ammonia production in the rumen (Morrison and Mackie, 1996). It is estimated that up to 74 % of protozoal biomass is recycled within the rumen (Ffoulkes and Leng, 1989).

Anaerobic fungi are more abundant when the diet has a greater percentage of fiber and may contribute up to 8 % of the microbial mass (Fonty and Joblin, 1991). Although it is not clear if the function of fungi in the rumen is important (Yokohama and Johnson, 1993), it has been found that they posses certain metalloproteases (Morrison and Mackie, 1996). In general terms, according to these last authors, mettalloproteases are present in fungi and bacteria, while cysteine- and serine-type proteases predominate in bacteria and protozoa and asparticproteases are present in protozoa.

2.1.2. Nucleic acids catabolism

Nucleic acids can be 5 to 9.5 % of the total N in grasses and hay (Smith and McAllan, 1970). These same authors observed that the additions of DNA and RNA pure or as plant material are rapidly hydrolyzed in the rumen. McAllan and Smith (1973) observed in ruminal fluid in vitro that purine nucleotides formed hypoxantine and xantine while pyrimidine nucleotides formed uracil and thymine. Some protozoa are able to take intact nucleic acids from the medium (Coleman, 1980). However, the microbial ecology of nucleic acid metabolism is poorly understood (Wallace and Cotta, 1997).

2.1.3. Urea and other nitrogenous compounds catabolism

Urea is broken down rapidly in the rumen yielding ammonia and when urea is fed may result in an overproduction of ammonia and an inefficient N retention (Morrison and Mackie, 1996; Wallace and Cotta, 1997). Urease, an enzyme with nickel content is mainly associated with the bacteria population allows the animal to break down urea either in the feed or from endogenous sources (diffusion and saliva) (Wallace and Cotta, 1997). However, when the concentration of urea is the same as blood or there is not a microbial population urea won't be hydrolyzed (Cheng and Wallace, 1979). All other aspects of urea metabolism are discussed in the section on whole body urea metabolism.

2.1.4. The rumen ammonia pool

There is an optimal ammonia concentration that is necessary for adequate microbial synthesis. However, this value will depend of the type of diet and the animal

requirements for a certain level of performance. Values reported have ranged from 5 to 23 mg/100ml (Febel and Fekete, 1996).

According to Leng and Nolan (1984) the ammonia pool is formed from many sources as follows:

- Protein, peptides and amino acids
- Miscellaneous soluble N compounds such as urea, uric acid, nitrates etc. Urea can be either recycled or in the feed
- Ammonia excreted from protozoa
- Gaseous N. Some atmospheric N_2 can be fixed by *Methanobacterium ruminatum* (.7g N per day in sheep, Li Pun and Satter, 1975).

The routes of ammonia losses can be by uptake by bacteria, outflow from the rumen, and ammonia absorption (Leng and Nolan, 1984). Ammonia absorption through the ruminal wall will be discussed in whole body N metabolism.

2.1.5. Microbial Protein and Amino Acid Synthesis

Not all the protein in the rumen is degraded totally to ammonia. When the diet contains true protein isotope studies have shown that 40-60% of the bacterial N can be incorporated without mixing with the ruminal ammonia pool, indicating that the microorganism have the ability to take up amino acids and peptides and/or deamination occurs within the organism (Ørskov and Miller, 1988). The amino acids are taken up as simple amino acids or as dipeptides. Wright (1967) found that peptide carbon is more efficiently converted into microbial protein than amino acid carbon and larger peptides were more likely to be incorporated than small peptides. Argyle and Baldwin (1989)

found that saturation of microbial growth occurred at 10 mg/L of added peptides and resulted in a higher growth than the corresponding amount of amino acids. These same authors found that the number of amino acids in a given mixture were more important for microbial growth stimulation rather than a sole amino acid being limiting.

Nitrogen assimilation by bacteria is also influenced by the ruminal ammonia level (Leng and Nolan, 1984). Bacteria assimilate at a high level of ammonia through the enzyme NAD-glutamate dehydrogenase or at a low level by NADP-glutamate dehydrogenase and these two are the most common mechanisms. There are two more mechanisms that bacteria can use when they are out of this range. Wallace (1995) and Wallace and Cotta (1997) summarized ammonia assimilation as follows:

- At levels of ammonia Km ammonia (mM) = 20 to 33 the principal mechanism is through NAD-glutamate dehydrogenase and it is the usual mechanism under normal circumstances

  α-oxoglutarate + NADH + NH_3 $\rightarrow$ Glutamate + NAD

- At high ammonia concentrations Km = 70 the enzyme acting is NAD-alanine dehydrogenase

  Pyruvate + NADH + NH_3 $\rightarrow$ Alanine + NAD

- At lower ammonia concentration Km = 1.8 to 3.1 the principal mechanism is through NADP glutamate dehydrogenase

  α-oxoglutarate + NADPH + NH_3 $\rightarrow$ Glutamate + NADP

- At Km concentrations of 1.8 there is also another mechanism through the action of glutamine synthetase- glutamate synthase

  Glutamate + ATP + NH_3 $\rightarrow$ Glutamine + ADP and then

Glutamine + NAD[P]H + α-glutarate → 2 Glutamate + NAD[P]

The assimilation of ammonia via NAD-glutamate dehydrogenase is the principal means of ammonia assimilation (Wallace, 1994). However, if ammonia concentration is low, efficiency of microbial growth is reduced because ATP is diverted from growth to the process of uptake of ammonia (Owens and Zinn, 1993).

The amino acid biosynthesis in bacteria also needs carbons to assimilate ammonia. Usually α-oxoglutarate is a central compound in aerobic organisms but in anaerobic organisms is obtained by partial Krebs cycle either forward or in a reverse direction depending of the bacteria (Wallace and Cotta, 1997). It is important for ammonia absorption both, the extra and intracellular pools, since for N fixation the extracellular N concentration should be high enough to maintain minimal intracellular concentration (Owens and Zinn, 1993). The ruminal ammonia concentration can be influenced by the diet, while carbohydrates degradation reduce it any dietary nitrogen usually increase it (Sauvant and van Milgen, 1995).

There are some special needs for certain bacteria that require certain type of amino acids. This is the case of cellulolytic bacteria that need branch chain volatile fatty acids in order to synthesize valine, isoleucine and leucine by reductive carboxylations and transaminations (Ørskov and Miller, 1988). However, little is currently known about the regulation of the enzymes of ammonia in this type of bacteria (Morrison and Mackie, 1996).

In protozoa there is also evidence of some de novo synthesis of amino acids but since protozoa usually utilize bacterial protein this mechanisms is of minor importance (Wallace and Cotta, 1997).

The biological value of the microbial protein leaving the rumen is approximately 66 to 87 compared with a value of 100 (Owens and Zinn, 1993). There are differences among bacteria nitrogen composition and these can be large. Although many of these differences may be explained by the different techniques used, still using the same techniques there are differences (Clark et al., 1992). Usually it has been observed that the nitrogen composition ratio nitrogen to diaminopimelic acid (DAPA) and N to purines are greater for the particulate associate bacteria than for the fluid associate bacteria (Clark et al., 1992).

Leaving the rumen we can have protein from the feed, microbial protein, amino acids, peptides, other non-ammonia compounds (NAN), and also ammonia. In addition to ammonia absorbed through the ruminal and omasal wall there has been demonstrated some peptide absorption in sheep such as Met-Gly and carnosine (Matthews and Webb, 1995). It is believed that some amino acid absorption occurs through the ruminal wall but the magnitude of this process was evaluated as insignificant (Webb and Matthews, 1994). However, the structure of the keratinized squamous epithelia makes this type of study difficult (Matthews, 2000).

In the abomasum, HCl and pepsin (an enzyme secreted here), which are part of the gastric juice, help to solubilize the protein and pepsin, starts cleaving some peptide bonds.

2.2. Nitrogen digestion and absorption in the small intestine

The small intestine is the major site of amino acid absorption. Once protein and amino acids reach the small intestine, the enzymes trypsin and chymotrypsin,

carboxypetidases A and B and elastases act on the proteins and yield oligopeptides, peptides and amino acids. These enzymes are secreted by the pancreas as proenzymes and are activated mainly by an enterokinase that is secreted in the wall by the mucosal epithelial (Breazile and Houghton, 2001). Although ruminants possess well developed protease activity at birth, in a review Harmon (1993) suggest that the young calf is more sensitive to nutritional modification of digestive enzymes (mainly pancreatic proteases) than are fully developed ruminants.

Once protein is digested to amino acids they are absorbed through a system of transporters. Usually these transport system have low affinities for substrates that have large capacities for transport, whereas those that display high affinities have low capacities for transport (Matthews, 2000). These transporters have been identified in selected tissues, cells and apical and basolateral membranes (Matthews, 2000). In the small intestine, there are at least five Na^+ dependent cotransports systems and two independent Na^+ coports in the microvillous (luminal) membrane of the intestinal absorptive epithelial cell (Breazile and Houghton, 2001). According to these authors there also has been identified two Na^+ dependent cotransport systems and three independent Na^+ coport transporters for amino acids in the basilateral membranes of absorptive epithelial cells. Reviews describing amino acid transporters in more detail can be found in Deves and Boyd, (1998), Palancin et al., (1998) and Mathews, (2000).

In sheep it is believed that it occurs also in cattle the major site for amino acid absorption seems to be the ileum (Webb and Matthews, 1994). This absorption at the most distal part of the small intestine may have a physiological explanation and it is that the pH of the first two thirds of it usually do not increase to 7, pH at which the proteases

enzymes start to be more efficient in the small intestine (Ben- Ghedalia et al., 1974). According to Webb and Mathews (1994) there are many factors that may influence amino acid absorption in the small intestine:

- influence of the digestion process may have on the site of absorption
- varying affinities of the collective complement of transporters
- capacity of transporter for individual amino acid
- distribution of transporters along the small intestine (Deves and Boyd, 1998; Palacin et al., 1998).

Not all amino acids are absorbed at the same rate. Generally, the dietary essential amino acids tend to be absorbed in greater amounts when considered on a percentage basis (Webb and Matthews, 1994). In most of the experiments studying amino acid absorption, methionine was the amino acid removed in larger quantities (Christiansen and Webb Jr, 1990b; Christiansen and Webb Jr, 1990a; Mac Rae and Ulyatt, 1974). There are also interactions between amino acids that affect their final absorption of them. Infusion of leucine to the sheep abomasum suppresses the appearance of lysine to the portal blood (Hume et al., 1972). Methionine was observed to diminish and limited absorption of valine, leucine, alanine, glycine, lysine and phenylalanine by the brush border cells (Moe et al., 1987; Phillips et al., 1979). However, not all relations are antagonist. The infusion of leucine increased the appearance of methionine in the portal blood of sheep (Hume et al., 1972) and methionine increased absorption of threonine when the last was in small concentrations (Phillips et al., 1979).

In some cases di and tripeptides are absorbed and are finally converted into amino acids by the brush border cells (Harmon, 1993; Webb et al., 1993; Breazile and Houghton, 2001). Results showed that peptide absorption may be largely by non mediated processes, but mediated processes are also present (Webb, 2000). The absorption of peptides may have an energy advantage when compared to amino acid alone. The absorption of peptides independent of carriers may occur by the envelopment by membrane vesicles (endocytosis) or by diffusion through existing membrane spanning channel proteins or paracellular pathways (Webb and Matthews, 1994). According to Webb and Mathews (1994), this type of peptide absorption is effected by extra and intra cellular peptide concentrations, by the signals affecting membrane endocytosis, by structural protein function, peptide size and charge relatively to channel protein size and charge and by the energy inside the cell.

In ruminants the effect of nitrogen intake show that the relation between net portal flux of amino acids and nitrogen intake is not as strong when compared to the ammonia N flux and nitrogen intake (Seal and Reynolds, 1993) mainly if these forms of nitrogen are highly digestible (Reynolds et al., 1991). It appears that the intake of metabolizable energy will predict in a better way the portal amino acid flux rather than nitrogen intake (Reynolds et al., 1994). This could be associated mainly with an increase of microbial protein that reaches the duodenum (Hoover and Stokes, 1991).

Tagari and Bergman (1978) found that there is a quantitative imbalance between amino acids disappearing from the gut and amino acids appearing in the portal blood in sheep. This observation has also been confirmed in cattle. Most of the glutamine and

almost all the glutamate and aspartate in the diet are catabolized by the small intestine intestinal mucosa and CO_2 accounts for 56-64% of the metabolized carbons in non ruminants (Wu, 1998). Glutamine is readily used for energy in the gut, supply of amide nitrogen for purine and pyrimidine synthesis (Seal and Parker, 2000). The use of a glutamine isotope for intestinal protein accounted for 73 % of the gross flux of amino acids across the small intestine of lambs (Gate et al., 1997).

The portal drained viscera (PDV) uses more glutamate and glutamine than it is available from dietary sources (Reynolds and Huntington, 1988). These amino acids are oxidized and their amino groups are transmitted to form alanine, serine and glycine (Bergman and Pell, 1984).

2.3. Whole Animal Nitrogen Metabolism

The liver controls the fate of the nutrients absorbed to the peripheral tissue through its central position and has an important function in detoxifying the organism of useless and/or substances that appear in large quantities. This role in nutrient partitioning (homeorhesis) of the liver is very important by the involvement in regulating insulin, producing growth factors (IGF-I), and removing from the blood of hormones (Lobley et al., 2000). In order to discuss nitrogen metabolism I will separate in ammonia and urea; protein and amino acids; and nucleic acid and hormonal control.

2.3.1. Metabolism of Urea and Ammonia

The metabolism of ammonia and urea in ruminants has been reviewed by Milligan and Kennedy (1980), Visek (1984), Parker et al., (1995) and Huntington and Archibeque (1999).

Physiologically ammonia in the rumen is as ammonium (NH_4) but it is absorbed as NH_3 so as ruminal pH increases absorption of NH_3 does as well. The amount of ammonia in the rumen reflects the solubility and fermentability of the dietary and endogenous sources of N (Huntington and Archibeque, 1999). Most forages have larger amounts of soluble nitrogen although it can range from 60 to 100 %. This increases the amount of ammonia in the portal blood depending of the liver to detoxify it. Also grains have a wide range of soluble nitrogen that may be affected by the processing. In growing beef cattle fed diets high in rumen soluble nitrogen, increase ammonia absorption by the portal blood viscera has been associated with net removal of amino acids by liver (Reynolds et al., 1991). Although the mechanism for this amino acid removal is unclear, it has been suggested that it may be due to an increase need for amino acid N requirement in transamination reactions to generate glutamate and aspartate which cannot be met by mithocondrial capture of ammonia as glutamate (Reynolds, 1992). Under conditions of high urea flux, the mitocondrial supply of NH_3 may not be sufficient inducing the obligatory use of amino acid nitrogen to maintain urea synthesis (Seal and Parker, 2000). It seems that under excess of ammonia and an increase of urea synthesis, the liver will be competing for amino acids with the rest of the organs and tissues for amino acids. In beef steers fed either an alfalfa based diet containing 17 % CP or a concentrate based diet with 12% CP at equal levels of metabolizable energy, net PDV absorption of α-amino

nitrogen was similar to both diets but net PDV absorption and liver removal of ammonia was more than doubled when steers were fed the alfalfa diet compared to the concentrate diet (Huntington, 1990). This increase in ammonia removal by the liver resulted in a doubling of urea removal by the liver, and resulted in a threefold increase in α-amino acid nitrogen removal which markedly reduced splachnic release of these amino acids for the other body tissues (Reynolds, 1992). A similar response was observed in beef heifers fed isonitrogenous diets with different concentrate to forage diets ratios (Reynolds et al., 1991). Continuing this line of thinking animals fed low quality forages diets with urea could the ammonia absorbed and removed by the liver be competing or reducing amino acids to the rest of the tissues and partly explain low responses when given urea to cattle with low quality forages diets?

Ammonia in the ruminant as in other mammals does not come only from what is absorbed in the diet. There is a net production of ammonia N by the PDV that can range from 16-95% of N intake and it is directly related to N intake (Huntington, 1990). Extrahepatic tissues such as muscle may produce free ammonia (Van Der Walt, 1993).

The blood arriving to the liver comes from the hepatic portal vein and the hepatic artery, although this last one is believed to make only a small contribution (Reynolds, 1995). The blood flow throughout the PDV is highly and positive correlated with their metabolic energy intake (ME) (Huntington, 1990). In the liver, periportal and perivenous cells have the enzymes of the ornithine cycle to form urea and to use glutamine synthesis for detoxifying the organism of ammonia (Meijer et al., 1990). The capacity of the hepatocyte to detoxify NH_3 directly to urea appears to be well adapted to large changes in portal NH_3 concentration being efficient in the removal at normal values usually found in

common diets (Meijer et al., 1999; Symonds et al., 1981). These cells contain also amino-acid degrading enzymes. A glutamine synthase system probably functions as a pericentral scavenger in order to eliminate any ammonia that might have escaped the urea cycle (Meijer et al., 1999). This system is a high affinity, low capacity mechanism while the urea cycle may be described as a low affinity, high capacity mechanism (Van Der Walt, 1993). It has been suggested that the major factors affecting the rate of ureagenesis in the liver are the intramitocondrial concentration of ammonia and the concentration of N- acetylglutamate (Van Der Walt, 1993). It has been hypothesized that pH is the major factor that determines the balance in the glutamine cycle, where glutamine production predominates at low pH and urea production predominates at high values (Van Der Walt, 1993).

The urea formed in the liver goes into blood and may be removed by the kidney or may be recycled to the rumen through saliva or the blood as well as other regions of the digestive tracts (Huntington and Archibeque, 1999). Urea may recycle to the rumen through two ways by saliva or by blood across the rumen wall. It is calculated that up to 70 % of the N secretion of the parotid gland may be urea recycling (Breazile and Houghton, 2001). This N recycling back to the rumen allows the ruminant to be able to live with lower amounts of nitrogen in the diet. The lower the nitrogen in the diet the higher percentage of N is recycled (Owens and Zinn, 1993). The principal factors affecting the rate of endogenous urea transfer from the blood to the lumen of the gastrointestinal tract are the organic matter digestibility, plasma concentration of urea, and ruminal ammonia concentration being the first two positive and the last one negative related (Kennedy and Milligan, 1980). Huntington and Archibeque (1999) stated that

some other factors may also be involved in urea transfer such as capillary blood flow and CO_2 tension.

The diet composition affected the site of urea N flux across the PDV of steers, when fed hay urea was transformed mainly to the post stomach, when fed concentrates these same steers urea flux shifted to the rumen (Huntington, 1990). Urea recycled to the gut, production and excretion is linked to diet composition, intake and productive priorities of the animal and from 19 to 96% of endogenous production may be recycled to the rumen (Huntington and Archibeque, 1999).

Seal and Parker (1996) demonstrated that the volatile fatty acid pattern in the rumen influences the N metabolism and the amino acid flux to the portal vein. Additional energy also reduces the proportion of urea-N in urine when the N supply increased (Huntington and Archibeque, 1999; Parker et al., 1995).

2.3.2. Amino acid, Protein and Nucleic acid metabolism

The absorbed amino acids absorbed are carried by the portal vein to the liver. The PDV uses amino acids from dietary and endogenous sources (Tagari and Bergman, 1978) using more glutamine and glutamate than are available in the diet (Harmon and Avery, 1987). These two amino acids are oxidized and the amino groups are transformed to form alanine, serine and glycine (Bergman and Pell, 1984). However, in the liver there is a net uptake of glutamine and an output of glutamate (Bergman and Pell, 1984; Reynolds, 1992). In experiments where urea was added to ruminant diet, an increased hepatic NH_3 uptake was seen, but glutamine uptake was either unchanged or slightly increased (Maltby et al., 1991). Glutamine and glutamate are interconvertible and the amount of

glutamate transported depends on the quantity of the first (Bergman, 1986). In most species, glutamines appears to be the more abundant amino acid in tissues (Lobley et al., 2001). The amino group of glutamine is important in ammonia detoxification and in acid base balance and being the more common free amino acid ensures that this is the probable amine donor (Lobley et al., 2001). Glutamine appears to act as a nitrogen carrier between the kidneys, muscles, liver and PDV and it is also used to neutralize acids produced during the acidosis resulting from fasting (Bergman and Pell, 1984). This shuttle of glutamate and glutamine between liver and peripheral tissues provides a mechanism for transporting ammonia in a safe way to be detoxified by the liver via ureagenesis (Reynolds, 1992).

Gluconeogenic amino acids such as glycine and alanine are absorbed in the greatest amount by the PDV but even larger amounts are removed from the liver (Bergman, 1986). This is important since free amino acids are major players in the interorgan exchange of carbon and nitrogen in ruminants mainly between peripheral tissues and the liver (Reynolds, 1992). Alanine has been found to be removed also by the kidneys apparently for gluconeogenesis, but in the hindquarters alanine was consistently released in sheep (Brockman and Bergman, 1975). Also glutamine, glycine and arginine are important in the transport of amino groups derived from deaminated amino acids in muscle (Bergman, 1986).

The liver removes arginine and it is released as citrulline and ornithine, but in contrast the kidney and hindquarters released arginine and removed citrulline and ornithine (Bergman, 1986).

Most of the amino acids are removed in a net basis by the liver with exception of glutamate as previously explained and the branched chain amino acids (BCAA) that are usually released by the liver (Bergman, 1986; Seal and Reynolds, 1993). The BCAA have the lowest rates of fractional removal, under a wide range of physiological and nutritional conditions, in both cattle and sheep (Lobley et al., 2000). The capacity of the liver to remove BCAA is limited and extrahepatic tissues must carry out the further removal (Lobley, 1992).

The removal of amino acids by the liver is important since this organ is a major site of glucose, protein or urea synthesis and the peripheral tissues are major sites of glucose and protein turnover (Seal and Reynolds, 1993). According to Wray-Cahen et al. (1997) there are four possible fates of the absorbed amino acids in the liver and these are (i.) retention in the free form, (ii.) conversion to specific metabolites, (iii.) oxidation and (iv.) incorporation into hepatic and export proteins.

Generally the nonessential amino acids are removed in excess to essential amino acids and results in a total splachnic release for glutamate, lysine and BCAA (Reynolds et al., 1994). In the fasted ruminant the BCAA leucine that is released from muscle turnover is used by the liver contributing towards protein synthesis rather than acting as an oxidative substrate (Pell et al., 1986).

All the tissues have common energy dependent maintenance processes that are protein turnover, substrate cycling, and ion transport (Harris and Lobley, 1991). The PDV and liver have a high protein turnover by themselves. In general, amino acids use by the PDV are related to the high rate of protein synthesis in the PDV (Lobley et al., 1980). The liver has a large metabolic oxygen consumption accounting for 18-26% of whole

body oxygen consumption of beef heifers (Reynolds et al., 1990). The liver increases the consumption of oxygen as intake increases at a greater rate than the PDV and it is not reduced during fasting as markedly as the PDV (Reynolds, 1995).

Many metabolic processes are carried out by the liver, among them protein synthesis. In sheep and cattle the liver can account for 9 to 13% of body protein synthesis (Davis et al., 1981; Eisemann et al., 1989). The hepatic protein content is determined by the relative rates of protein synthesis and degradation and the export of protein to extracelluar fluid (Meijer et al., 1999). According to these authors turnover of liver proteins rapidly responds to the increase in amino acid supply during and after meals by an increase in protein synthesis and a decrease in the rate of protein degradation while in fasted animals the opposite occurs.

Whole body protein turnover per unit of body weight in the adult mammal is inversely related to metabolic body size (Buttery, 1984). In well-fed animals protein degradation in muscle and other tissues is similar in magnitude to that of protein synthesis (Harris and Lobley, 1991).

Nitrogen arising from the catabolism of peripheral tissues is carried to the liver as alanine, glycine or glutamine for urea synthesis avoiding excessive release of ammonia (Seal and Reynolds, 1993). Amino acid flux through the body is very important and this flow from protein turnover exceeds that of intake by two to threefold while N at maintenance, amino acid catabolism is 6 to 10% of flux but still is equal to net absorption (Harris and Lobley, 1991).

The skin utilizes as much glucose as muscle and a lower amount of acetate per unit of weight compared with muscle, however, both tissues have different requirements

for oxygen since a significant proportion of glucose is degraded anaerobically as part of the lipogenic demand (Harris and Lobley, 1991). Due to anaerobic metabolism of epidermis lactate is produced (Breazile and Houghton, 2001). The gut and skin represent almost 50 % of total protein synthesis on a daily basis but only contribute to 10 % of total body accretion, meanwhile muscle values are approximately 17 % for protein synthesis and 36% for protein accretion (Seal and Parker, 2000).

2.4. Hormonal Control of Nitrogen Metabolism

The nitrogen metabolism in the body is controlled in part by hormones, which respond to different signals and regulate the flow of nutrients among tissues. In ruminants not all the tissues respond to all the hormones. The ability of tissues to respond to hormones may vary with physiological age, nutritional, and pathological state and there can be changes in responsiveness due to sensitivity (Kahn, 1978).

Generally it is considered that there are two types of control in the body for the regulation of nutrient partitioning, homeostasis and homeorhesis. The homeostatic control acts so that despite challenges from the external environment the internal environment remains relatively unchanged. In the short term after a meal the homeostatic control exerted by insulin and glucagon results in a relatively constant supply of nutrients to the peripheral tissues by promoting the storage of nutrients and the mobilization of these in the postabsorptive period (Bauman and Currie, 1980).

Homeorhesis is the integrated changes for the priorities of physiological state that occur in nutrient partitioning for processes such as growth, pregnancy and lactation (Bauman et al., 1982; Bauman, 2000; Bauman and Currie, 1980). Homeorhetic

adaptations, therefore, allow for chronic alterations or even redirection of physiological processes while still allowing homeostatic systems to preserve constant conditions constant (Bauman, 2000). Homeorhetic controls have been cited in processes such as puberty, ageing, chronic undernutrition, chronic illness, hibernation, premigration and migration, egg laying, incubation anorexia, seasonal cycles and exercise (Bauman, 2000).

During the increase demand for lactation and growth, feed intake increases and changes are seeing in the liver and intestinal epithelial size increases. The supply of nutrients to such tissues is dictated in turn by the availability of nutrients, nutrient uptake by the tissues, activity by the tissue enzymes and blood flow (Bickerstaffe, 1993).

The principal hormones that interact in nitrogen metabolism are discussed briefly.

2.4.1. Insulin and nitrogen metabolism

Insulin acts on a variety of tissues and alters a variety of processes as enzyme activity and enzyme amount (Vernon and Sasaki, 1991). It also stimulates the transport of glucose into the muscle, and protein and amino acid sequestration in all target issues (Berne and Stanton, 1998). In cattle and sheep a raise in insulin concentration increased the glucose arteriovenous difference across the hind limbs, which could be to increase glucose uptake by skeletal muscle (Vernon and Sasaki, 1991). If it is administered in a basal state, insulin lowers the plasma of all amino acids and after a protein meal insulin secretion rises and limits the essential BCAA in blood (Berne and Stanton, 1998).

In the liver, insulin rapidly inhibits glycogenolysis and therefore glucose output and also inhibits gluconeogenesis. This last inhibition is accomplished by decreasing the

hepatic uptake of precursor amino acids and their availability from muscle (Berne and Stanton, 1998).

Both protein anabolism and glycogen storage of glucose require at cellular level that insulin exerts an effect on the transport of glucose, phosphate, monovalent cations and amino acids transport into cells (Messina, 1998). This hormone even in those cells in which it has no direct effect on DNA synthesis can also induce protein synthesis and it also inhibits protein degradation (Messina, 1998). According to this author insulin can also regulate a number of genes whose products are associated with cell differentiation or cell proliferation.

The response to insulin varies with the physiological state (growth, pregnancy, lactation), diet and type of tissue. This different responsiveness to insulin in the ruminant is part of the orchestrated changes that occur in the body in response to the above physiological states and diet and is called homeorhesis. During late pregnancy there is insulin resistance with respect to glucose utilization in the whole animal (Hay et al., 1988). In the onset of lactation there are some responses to insulin that are attenuated such as gluconeogenesis inhibition (liver), fat synthesis (adipose tissue), glucose uptake (muscle), and glucose oxidation (whole body) (Bauman, 1984; Bauman, 2000; Vernon and Sasaki, 1991). During early lactation, there is almost no response to insulin for glucose and acetate uptake by the adipose tissue, and also by the hindlimb where the responsiveness to insulin is substantially reduced but not the sensitivity to insulin reducing glucose uptake (Bauman, 2000). However, the complexity of endocrine control of nutrient partition is likely to be complex with overlapping systems involving many synergisms (Bauman and Currie, 1980). Homeorhesis is also believed to occur during

growth were nutrients are first channeled to the central nervous system, bone, muscle and fat, respectively, at different ages from conception to maturity (Bauman, 1984). In older animals there is almost no response to insulin in muscle protein synthesis, showing a chronological attenuation (Nieto and Lobley, 1999).

Nutrition affects hormone release and their receptors acting the body different according to the signal received. Fasted animals have a greater response to insulin that animals well fed (Nieto and Lobley, 1999). There is some evidence that the insulin to glucagon ratio may be determining the amino acid partitioning between the liver and the peripheral tissues in the transition from fasted to fed (Lobley, 1994). According to Lobley (1994) insulin will be more sensible to be the one to change the ratio insulin to glucagon since the latter is much less sensitive to intake. Feeding rations with high amounts of concentrates can change the partitioning of energy in ruminants away from milk production towards body gain in a mechanism believed to be related to an insulin peak after a meal (Hart, 1983).

Insulin action may also be influenced by the BCAA, mainly leucine which may act as a possible signal of nutrient availability to peripheral tissues (Lobley, 1998). In conditions of supramaintenance protein metabolism may be more influenced by the growth factor – insulin like growth factor I axis (Nieto and Lobley, 1999)

2.4.2. Growth hormone (GH) and nitrogen metabolism

There are several reviews of growth hormone action in tissues (Bauman and Vernon, 1993; Berne and Stanton, 1998; Breier and Sauerwein, 1995; Burton et al., 1994; Etherton and Bauman, 1998; Müller et al., 1999; Pell and Bates, 1990)

Growth hormone is an anabolic hormone, stimulates cell division, skeletal growth and protein synthesis and it is also lypolitic (McDowell and Annison, 1991). Growth hormone increases bone growth and decreases protein degradation (Breier, 1999) and it is also diabetogenic (Hart, 1983). Growth hormone increases nitrogen retention in animals given this hormone and there was seen a decreased excretion of urine nitrogen and α-amino acids in cattle (Eisemann et al., 1989; Etherton and Bauman, 1998). Eisemann and coworkers (1989) also reported an increase in whole body protein synthesis and a reduction of leucine oxidation. In dairy cows there has been reports of increasing milk protein with exogenous GH (Etherton and Bauman, 1998).

Nutritional status has a major role in determining circulating GH concentrations and is elevated as a result of undernutrition (Breier, 1999). According to Breier (1999) high concentrations are the reflection of the influence of factors such as hypoglycemia, stress, and low serum free fatty acids on the pituitary secretion of GH. However, also GH blood levels increase after a high protein meal or the infusion of a mixture of amino acids, being arginine the most consistent amino acid stimulator (Berne and Stanton, 1998).

Many of GH actions are carried out by the peripherally generated somatomedins and the insulin like growth like factors IGF I and IGF II either locally or systemic (Nieto and Lobley, 1999; Owens et al., 1993).

2.4.3. Insulin Like Growth Factor (IGF-I and IGF-II)

Various tissues synthesize insulin like growth factors, which are a family of polypeptide hormones related to insulin, and it is believed they act through autocrine,

paracrine, and endocrine mechanisms (McMurtry et al., 1997). The IGF's are released after the GH target certain tissues such as liver and muscle and stimulate among other function protein synthesis. The GH receptor (GHR) is present in high concentrations in liver and in lower concentrations in muscle, growth plate, fat, heart, kidney, brain and placenta (Breier and Sauerwein, 1995). The interaction between GH and the GHR initiate the secretion of IGF, which in turn promotes proliferation and differentiation (Breier and Sauerwein, 1995).

In a series of experiments it has been found that the plasma concentration of IGF-I correlates well with the growth rate of young animals given diets with proteins of various nutritional values and with the rate of whole body protein synthesis (Dawson et al., 1998; Noguchi, 2000). Growing steers kept at maintenance showed a marked reduction in GH binding to hepatic membranes, elevated GH secretion and a fall in IGF-I levels and no changes of this level even through intravenous GH administration (Breier and Sauerwein, 1995).

Apparently, the current data shows that the IGF action on muscle is via an endocrine rather than an autocrine or paracrine mechanism (Lobley, 1998). Most of the growth responses of IGF-1 is by the receptor (IGF-1R) which shows a close relation to the insulin receptor with a similar subunit structure and some immunological determinants in common (Breier and Sauerwein, 1995).

Insulin like growth factors are carried in blood by binding proteins (IGFBP) and are recognized till now six different types of IGFBP which apparently may have other biological functions than carrying IGF such as cell growth, modification of cell bone proliferation, and growth arrest of breast and prostate cancer cells (Hwa et al., 1999). As

said above reduced nutrition reduce affinity of GHR and a reduction of IGF-I concentrations, however, relative changes in IGFBPs concentrations and tissue specific alterations in IGF-I receptor concentrations may sustain the availability of IGF-I for those tissues that are critical (Breier, 1999).

The relations among GH, IGF-I and insulin suggests that in the fasted animal, where both insulin and IGF-I are low, muscle protein degradation will be elevated and synthesis will be suppressed but as intake increases over maintenance with adequate protein levels insulin will cease to exert any major additional effect on protein metabolism and GH/IGF-I will stimulate synthesis and degradation but the latter at a lesser extent (Lobley, 1994).

2.4.4.. Other Hormones

There are other hormones that may act by themselves or by facilitating the action of insulin, GH and IGF-I either by acting on tissues and/or nutrient availability.

Prolactin is very important during the onset of lactation by altering the nutrient partitioning, synthesis of milk, and maintenance of milk secretion (Freeman et al., 2000). Prolactin has been reported to increase nitrogen retention in several species (Bauman et al., 1982). It increases mammary tissue growth (Knight, 2000), and as a result of binding to its receptor induces transcription of genes for the milk proteins casein, lactoalbumin and β-lactoglobulin and also stabilizes their mRNAs (Berne and Stanton, 1998).

Glucagon has mainly the opposite action of insulin, however, it doesn't act over the mammary gland. It increases glucose free fatty acids and ketone bodies in the blood. Its ratio with insulin is more important than its concentration by itself. If a protein meal

is fed, insulin secretion increases, preventing unneeded proteolysis and facilitating amino acid intake but at the same time glucagon secretion increases preventing the decrease in hepatic glucose output (Berne and Stanton, 1998).

Epinephrine and norepinephrine increase lipogenesis, glucose concentration in blood, and the use of glycogen by the muscle.

Glucocorticoids indirectly facilitate the action of other hormones (Vernon and Sasaki, 1991). Cortisol also maintains glucose production from protein, facilitates fat metabolism, modulates central nervous system and profoundly affects the immune system (Berne and Stanton, 1998).

3. Phosphorus physiology in the ruminant

Phosphorus is the major anion of intracellular fluids and the second most abundant mineral found in the animal body. About 80 % of the body phosphorus (P) is in the skeleton (bone and teeth) with the remaining 20 % in nucleotides such as ATP, nucleic acids, phospholipids and other phosphorylated compounds involved with metabolism and in maintaining the acid-base balance of body fluids (NRC, 2001; Soares Jr, 1995).

3.1. Ruminal metabolism of phosphorus

Ruminal microorganisms require P for their growth and cellular metabolism (NRC, 1996; Ternouth et al., 1985). Microorganisms also required P for the digestion of cellulose and for the synthesis of microbial protein and volatile fatty acid production (Breves and Schröder, 1991; Burroughs et al., 1951). The recommended available

phosphorus in order to optimize degradation of cell walls by the microbes from dietary sources and salivary recycling in the rumen should be at least of 5 g/kg of organic matter digested (Durand and Komizarczuk, 1988). The optimal concentration in the rumen for having undisturbed microbial metabolism can range from 0.7 to 2.6 mM (Breves and Schröder, 1991).

Much of the phosphorus in grains is in the form of phytic acid, which is a problem for non-ruminants but not for ruminants and is only produced and stored during seed production by the plant (Marschner, 1995). Most of the data published suggest a complete hydrolysis of phytate by rumen microbes and almost the same bioavailability than inorganic P (Pi) for ruminants (Soares Jr, 1995).

There is some controversy if P and Pi are absorbed from regions of the digestive tract cranial to the duodenum. The results of these studies differ greatly; some workers didn't find either absorption or secretion, while some others found net absorption or net secretion. Injection of the tracer ^{32}P into the rumen or intravenously showed that the rumen epithelium was permeable to Pi in both directions, but only in insignificant amounts (Ternouth et al., 1985; Ternouth, 1997; Yano et al., 1991).

3.2. Digestion and absorption of P in the small intestine

The absorption of P takes place, as with Ca, in the duodenum by both active and passive absorption (Braithwaite, 1984; Kincaid, 1993; Wasserman, 1981). No matter how P is ingested its absorption will depend on its solubility at the point of contact with the absorbing membranes (Braithwaite, 1984; Maynard et al., 1979). Phosphorus absorption also is influenced by intestinal pH, animal age, and intake of calcium (Ca), iron,

aluminum, potassium (K) and magnesium (Mg) (Braithwaite, 1984; Hays and Swenson, 1993; Mc Dowell, 1992; McDowell, 1992). The low digesta pH in the abomasum results in P to be found in a soluble form in the duodenum and the jejunum which facilitates the absorption (Ternouth, 1997). Phosphorus is absorbed in the ortho phosphate form (Kincaid, 1993). Absorption of P is further stimulated by a pH gradient across the brush border membrane (Breves and Schröder, 1991; Care, 1994; Yano et al., 1991). When the supply of P exceeds requirement the efficiency of absorption is reduced (Care, 1994). Phosphate absorption is increased by vitamin D_3 which may change membrane permeability, alter configuration of a phosphate carrier, stimulate pump sites, or by Ca absorption, indirectly increasing P absorption by decreasing the degree to which P is insolubilized by Ca (Kincaid, 1993).

3.3. Whole body metabolism and hormonal control of phosphorus

The levels of inorganic P in plasma are not under strict homeostatic control as with respect to Ca (Kincaid, 1993). Plasma Pi concentration is normally between 1.3 and 2.6 mmol/L or 4 to 8 mg/dL (Goff, 2000). Unlike non ruminants in which the kidney plays a fundamental role in P homeostasis, in ruminants the kidney's role is not as important because saliva and endogenous fecal loss help to keep P levels in place. Saliva is an additional source of P for the rumen with concentrations ranging from 370-720 mg/liter in mixed saliva, much higher concentrations than that found in plasma (60 mg/liter) (Yano et al., 1991). In dairy cows between 30 and 90 g/d of phosphorus is secreted daily into saliva (NRC, 2001).

The factors that affect P secretion of saliva are the time spent ruminating (chewing activity) and the parathyroid hormone (PTH) status of the animal (Goff, 2000). There was also found a relationship between physical form and absorptive efficiency of P, with higher efficiencies for unchopped hay compared with chopped hay (Yano et al., 1991). This is probably explained by the higher volume of saliva produced by the animal eating unchopped hay, and this has also been seen in dairy cows on diets containing more neutral detergent fiber (NDF) (Khorasani et al., 1997) which resulted in both cases in more salivary P and more P available in the duodenum (Yano et al., 1991).

The action of PTH is to regulate mainly calcium metabolism, maintaining its level in blood through its actions in bone (increases resorption) and on the kidney (increases reabsorption) being its secretion is inversely related to calcium blood levels but independent of plasma phosphate levels (Scott, 1986). Parathyroid hormone increases renal and salivary excretion of P and this can be a reason why hypocalcemic animals may tend to become hypophosphatemic (Goff, 2000). In ruminants P depletion does not induce significant changes of plasma $1,25(OH)_2D_3$ and didn't affect V_{max} of Na^+-linked Pi uptake across jejunal brush border of growing goats (Breves et al., 1995). However, the V_{max} of the H^+/Pi cotransport mechanism increase to P depletion in sheep proximal small intestine (Breves et al., 1995). For Goff (2000) the secretion of 1,25-dihydroxyvitamin D can be secreted in response to low plasma P levels, but this must be very low (less than 1 or 2 mg/dL). More research is needed in order to understand the effects of P depletion at the cellular level in ruminant species.

Parathyroid hormone stimulates 1,25-dihydroxyvitamin D secretion by the kidney increasing phosphate absorption in the small intestine, however, PTH is secreted in response to hypocalcemia and not hypophosphatemia (Goff, 2000).

Phosphate absorption is increased by vitamin D_3 which may change membrane permeability, alter configuration of a phosphate carrier, stimulate pump sites, or by Ca absorption, indirectly increase P absorption by decreasing the degree to which P is insolubilized by Ca (Kincaid, 1993).

Calcitonin, a hormone secreted by the thyroid glands exerts hypocalcemic influence by inhibiting osteoblastic bone resorption and urinary P reabsorption at the renal tubule in monogastric animals (Yano et al., 1991). However, its function in ruminants its not totally clear.

In ruminants, P is excreted mainly in the feces. When plasma P level is high, 2.0 to 2.5 mmol/liter, the kidney will excrete P (Challa and Braithwaite, 1988). When high concentrate diets are fed, more P is excreted in the urine of cattle (Mc Dowell, 1992) or when diets contain no long fibrous materials requiring rumination (Ternouth, 1997).

4. Potassium physiology in the ruminant.

The major cation in intracellular fluid, potassium is involved in the regulation of osmotic pressure, water balance, muscle contraction, acid-base balance, nerve impulse transmission, and certain enzymatic reactions (Miller, 1995). Potassium also is important in the transport of oxygen and carbon dioxide through blood, being responsible for at least half of the carbon dioxide capacity of the blood (Mc Dowell, 1992). Potassium helps to maintain the electrical neutrality before buffering of hydrogen ions by hemoglobin in

blood, by keeping an ion balance with the carboxyl groups (Reece, 1993). After ionization of carboxyl groups is suppressed by hydrogen ions, electrical neutrality of K ions is maintained by bicarbonate and chloride ions (Reece, 1993)

Potassium, as well as sodium, is a component of the ATP-Na-K pump, which maintains a concentration gradient important for the transport of substrates through the cell membrane, and for the regulation of the osmotic pressure (Mc Dowell, 1992).

Potassium is mainly absorbed from the rumen, omasum and the lower gastrointestinal tract (Mc Dowell, 1992). Absorption from the intestine is by simple diffusion (Ammerman and Goodrich, 1983). A large proportion of K in the rumen is derived from the saliva, which is continuously secreted and is rich in K (Mc Dowell, 1992).

Potassium balance depends mainly on the excretion by the kidneys, which adjusts K excretion rapidly and precisely to a wide variation of intake (Berne and Stanton, 1998). High Na intake may increase K urinary excretion (Ammerman and Goodrich, 1983). Adrenal hormones including aldosterone increase potassium secretion by the renal tubules (Mc Dowell, 1992) while increasing Na absorption (Ammerman and Goodrich, 1983). Extracellular fluid potassium concentration is regulated precisely at about 4.2 ± 0.3 mEq/liter (Berne and Stanton, 1998). According to these authors, precise control is necessary because many cellular functions are dependent on extracellular potassium concentration.

5. Supplementation of high quality forages

Supplementation of forages has been reviewed by several authors (Horn and McCollum III, 1987; McCollum III and Horn, 1991; Moore et al., 1999; Paterson et al., 1994) and several aspects included in these reviews will not be discussed here.

High quality forages are usually referred to some cool season grasses (CSG), legumes and/or the mixture of them. These high quality forages include grasses that have a photosynthetic cycle C_3 and legumes which are also C_3 plants (Nelson and Moser, 1994). Although legumes are C_3 they can be separated into cool and warm season types based on their adaptation to temperature (Nelson and Moser, 1994).

Usually CSG are of higher digestibility than warm season grasses (WSG) not only because of the chemical composition but also because of the proportion and the physical arrangement of forage tissues (Goetsch and Owens, 1987). Also warm season C_4 species have higher concentrations of structural polysaccharides (Buxton and Fales, 1994).

Within the same plant specie the most important factor affecting forage quality is herbage maturity (Buxton, 1996). However, the environment also affects forage quality such as soil fertility, season, geographical location, temperatures, water stress and management (Buxton, 1996; Minson, 1990; Nelson and Moser, 1994).

Usually C_3 species in most of the cases are richer in protein with respect to energy creating an inefficient use of N by the ruminant. Several authors have hypothesized relatively utilizing different ratios of energy (total digestible nutrients or digestible energy and protein) or relating energy (concentrates) consumption as a percentage of body weight in order to assess proper supplementation to ruminants.

Moore et al. (1999) reviewed 66 publications on 126 forages to estimate the effect of non-lactating cattle consuming forages. These authors reported that generally supplements decreased intake with improved forages but these types of forages had the greater response when supplemental total digestible nutrients (TDN) was > 60% and when supplemental CP intake was > .05% of body weight (BW). These authors also reported a decrease voluntary forage intake when supplemental TDN intake was > .7% BW, when forage TDN:CP was <.7% or when voluntary forage intake was > 1.75% BW.

Horn and McCollum (1987) reviewed the effect of energy supplementation of forages in ruminants and concluded in general that substitution effects (units change in forage intake per unit increase in concentrate intake) were more pronounced with increasing forage digestibility. However, these authors were more inclined to the concept that than rather a single curve for substitution, a family of curves existdepending on the nutrient requirement of the animal. They included other possible factors affecting substitution such as activity, physiological state, forage quality and availability. Horn and McCollum (1987) concluded that concentrates could be fed up to .5% of BW without causing large decreases in forage intake. Bowman and Sanson (1996) in a review of literature concluded that grain supplements up to .25% BW had minimal effect on forage utilization but over that negative effects become larger.

Several factors have been identified affecting substitution between forage intake when concentrates are fed such as reduction of cellulolysis, ruminal pH, microbial interactions, rumen microflora composition, rate of passage, lipid supplementation and potential metabolic effects (Horn and McCollum III, 1987; Mould, 1988; Palmquist, 1988).

However, it's not only the relation of concentrate to protein but also the chemical composition of these elements and where and how they will be degraded in the rumen. The Beef NRC (1996) shows that degradable intake protein (DIP) for most of the legumes and CSG is around 60 to 90 % of the crude protein (CP). In order to avoid this a ratio protein truly digested in the duodenum to energy (digestible energy MJ) was calculated by Egan (1977) with sheep and proposed if this ratio was greater than 7.5 supplementation would be better with readily available carbohydrates. Also TDN values may be misleading when calculating a ratio of these type. While soybean meal, barley and corn have TDN values of 84-87, 84 and 90%, respectively, total carbohydrates and nonsoluble carbohydrates of barley and corn approximately duplicate and triplicate the values of soybean meal (Sniffen et al., 1992).

These differences in what are protein and energy supplements may be chemically composed and the possible interactions between them and the animal affects forage utilization should be taken into consideration when designing supplementation (Bowman and Sanson, 1996). A better understanding not only of the chemical interactions in ruminants of different diet components but also of how they relate to the different physiological states of the animal is needed.

6. Feeding Frequency

Feeding frequency has been studied mainly as a form of synchronizing N and energy (protein and carbohydrates) for optimizing rumen microbial yield. As previously stated, before most of the CSG and legumes have a high DIP which imbalance the rumen having more availability of N than other nutrients at a given time. In one study with

grazed forages containing over 17% of CP more than 40 % of N consumed disappeared from the rumen reaching a suboptimal relation of N-organic matter (OM) to the duodenum (Owens and Zinn, 1993). Energy supplementation increases the capture of released N and increase microbial N flow from the rumen (Owens and Zinn, 1993).

The feed potential of roughages depends on the size and metabolism of two fractions, the soluble fraction and the insoluble but fermentable fraction, at the rate the latter is fermented and how quickly the unfermentable residues can be passed from the rumen (Ørskov, 1998).

In high quality forages the N is usually rapidly released in the rumen where microbes usually do not have enough energy to capture that N that is afterwards excreted by the animal reducing its nutrient utilization efficiency. Varying the source and degradability of nonstructural carbohydrates and UIP we can vary the amount of amino acids reaching the duodenum. Greater dietary concentrations of NSC have increased the utilization of ruminal ammonia (Hoover and Stokes, 1991). The result should be more microbial protein reaching the small intestine and more nearly meeting the requirements of high producing cattle.

Two types of what in the literature appears as feeding frequency will be discussed; the meal served with different frequency in a day (FF) and animals having a basal diet but the supplement fed in different frequencies (SFF).

In a review by Owen (1978) the increase of FF of totally mixed diets improved dry matter and N digestibility, mainly with poorer quality feedstuffs and decrease the diurnal variation in the rumen variation of ammonia and volatile fatty acids.

Ulyatt et al. (1984) fed chaffed alfalfa to wether sheep once a day or once every hour. An increase in the FF had a major effect on reticulo rumen pool sizes but did not affect apparent digestibilities nor partition of digestion of non nitrogenous constituents. Daily feeding increased N reaching the duodenum; however, N retention was significantly greater with FF, suggesting to the authors the possibility of more efficient tissue utilization by the animal.

Feeding frequency did not affect ruminal or total tract OM or cell wall digestion either of lambs fed fescue hay 2, 4, 6, 8 or 16 times daily (Bunting et al., 1987) or of rams using a FF of alfalfa silage with or without soybean meal of 1 or 4 times daily (Ruiz et al., 1989). Ruiz et al. (1989) found that diurnal variation of all the ruminal parameters were reduced.

Dairy cows fed from 2 to 8 times a day resulted in an increase in the molar percentage of acetate and a decrease in the concentration of lactate in ruminal fluid (Bragg et al., 1986). An increase in acetate should produce an increase in milk fat. In this same direction dairy cows being fed more frequently (2 vs. 6 times a day), milk production was not increased but percentage of milk fat was increased by .4-.5% in two experiments (Kaufmann et al., 1980).

Increasing FF more than one time a day didn't affect intake of dairy cows (Burt and Dunton, 1967; Robinson and McQueen, 1994; Robinson and Sniffen, 1985). However, intake and nitrogen retention was increased when 5 kg medium quality hay was offered more than once a day (2 or 3 times) to zebu bulls (Ikhatua et al., 1987).

Altering the frequency of supplement (SFF) has been studied mostly under range conditions with low quality forages and protein supplements. Early works from McIlvain

and Shoop (1962) with cottonseed meal showed no difference in variation of performance within groups of steers supplemented once, every three days and every week. Also using as supplement cottonseed meal, Hunt et al (1989) studied the effect of SFF of 12, 24 and 48 hr and an unsupplemented control in a metabolism trial and with lighter growing cattle. Supplementation increase dry matter intake and VFA, NDF and ADF disappearance in situ and concentration of volatile fatty acids were greater for supplemented than for unsupplemented control ($P<.05$), however, there were no statistical differences due to SFF. In the performance trial these same authors only found differences when compare supplemented steers vs control in average daily gain but SFF did not show any differences among the hours fed.

Beaty et al. (1994) in a series of two experiments were they tested a daily supplementation vs a 3 times a week supplementation using pregnant beef cows grazing dormant tallgrass prairie. They found that reducing the SFF to 3 times instead of daily increased ($P<.02$) winter weight loss through calving. In a metabolism trial these same authors observed a slight improvement by daily supplementation, however, as a final consideration they thought that SFF of three times a week was a viable practice to reduce cost labor and with minimal consequences in terms of cow overall performance.

Farmer et al. (2001) fed a high protein supplement (43% CP) 2, 3, 5 and 7 days a week. Increasing frequency of supplementation increased linearly forage OM intake, OM and NDF digestion and they concluded that forage utilization was improved by more frequent supplementation but there was not expected a large impact in animal performance due to SFF.

Similar conclusions with protein supplements were obtained with wethers (Bohnert et al., 2001a; Bohnert et al., 2001b) and with cows during the last third gestation (Bohnert et al., 2001b) in which the authors concluded that supplements of 20 to 60 % UIP can be used by ruminants consuming low quality forages without adversely affecting N efficiency and animal performance by supplementing it once every 6 days. Similar results obtained Wallace et al (1988) with yearling heifers grazing dormant rangeland forage supplemented with a cottonseed cake 1 or 3 times a week finding not difference in the growth rate nor breeding performance due to SFF.

Krehbiel et al. (1998) determined the effects of feeding frequency of a protein supplement (soybean meal) on bromegrass intake and net portal and hepatic flux of nutrients in Dorset ewes. They found that SFF of soybean meal supplementation may affect the pattern of nutrient absorption without affecting their net absorption.

Chase and Hibberd (1989) fed chopped low quality hay free choice and fed two levels of corn (1.4 or 2.0 kg day $^{-1}$) on a daily or in an every other day basis. Supplementation frequency did not alter hay OM intake but tended to decrease total and hay OM digestibility. Digestibility and DM intake were lower for the animals in the high corn supplementation. These authors concluded that maize supplements should be fed on a daily basis in small quantities. These results are in agreement with the results reported by Wallace et al (1988) when they fed heifers with a grain cube daily or twice a week that resulted in weight lost and pregnancy rate decreased.

Hart (1987) studied the effect of two forms of grain (whole vs ground) frequency of feeding (daily or alternate) at 3 different levels (13, 26 and 39% of diet DM) on the intake and digestibility of sorghum silage. Feeding grain on alternate days decreased

NDF digestibility for the 13 and 39 % grain diet. The digestibility of NDF was depressed more by feeding ground corn on alternate day. Feeding corn on alternate days decreased dry matter intake for animals fed the 26 and 39% diets.

Altering the frequency of feeding, up to once a week, of a high protein supplement mainly in beef cows fed a low quality forage does not appear to affect overall performance. Feeding grain infrequently in low quality forages may affect digestibility and therefore overall performance.

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CHAPTER III

EFFECT OF THE FREQUENCY OF SUPPLEMENTING CRACKED CORN ON ALFALFA HAY INTAKE, UTILIZATION, AND PERFORMANCE BY GROWING CATTLE

Abstract

Two trials were conducted to study the effect of frequency of cracked corn supplementation on intake, utilization and performance of alfalfa hay by growing cattle. In Trial I, eight ruminally cannulated crossbred steers (501.9 ± 29.6) in a replicated 4 x 4 Latin square were given ad libitum access to chopped alfalfa hay with no supplement (CONT) or with a cracked corn supplement fed at one of three frequencies: 0.5 % of body weight (BW) every day (24); 1.0% of BW every other day (48) or 1.5% of BW every third day (72) (all animal eat the same on a 6d basis).Total organic matter intake was not different due to supplementing corn but it increased linearly ($P<0.01$) as corn frequency increased. Total digestible organic matter intake ($P<0.05$) and total tract digestibility ($P<0.01$) was increased by supplementing corn. Frequency of supplementation decreased linearly ($P<0.01$) total organic matter and increased linearly ($P<0.01$) total tract digestibility as feeding interval decreased. Feeding cracked corn increased total tract apparent digestibility of ADF ($P<0.01$) and of NDF ($P=0.07$). Ruminal concentration of butyrate ($P<0.01$) and the acetate to propionate ratio ($P<0.05$) of ruminal fluid was decreased by corn supplementation. In Trial 2, 60 Holstein heifers (198 kg) were stratified by weight and assigned to the same four treatments with the

exception that hay was not chopped. After 90 d on trial, feeding supplemental corn increased average daily gain and final weight ($P<0.01$), and both decreased linearly ($P<0.01$) as time interval between supplement feedings increased for the corn treatments. These data suggest that supplementing alfalfa hay with corn grain increased intake of digestible OM and increased ADG but supplementing at less frequent intervals (2d; 3d) increased hay digestibility slightly, but reduced intake of hay OM markedly and thereby reduced intake of digestible OM, and ADG.

Introduction

Supplementation of high quality forages with grain usually increases daily gains (Horn and McCollum III, 1987), total organic matter intake (Elizalde et al., 1999a) and digestibility of organic matter (Norton et al., 1982). Feeding frequency with supplements have been studied for protein supplements (Beaty et al., 1994; Farmer et al., 2001; Krehbiel et al., 1998) and grain (Chase and Hibberd, 1989; Wallace et al., 1988) on low quality forages. Feeding frequency has also been studied with oscillating protein for grain diets with lambs (Cole, 1999) or as a tool for trying to synchronize carbohydrate and nitrogen requirements in the rumen within the same day (Michalowski, 2001; Robinson and McQueen, 1994). However, almost no reports on the effect of the frequency of grain supplementation on high quality forages are reported in the literature.

The objective of this study was to examine the effect of frequency (every 24, 48 or 72h) of feeding the same amount of cracked corn (on a 6d basis) on intake, digestibility, ruminal parameters, and performance of cattle fed alfalfa hay ad libitum.

Materials and Methods

Experiment 1: Metabolism Trial

Animal and Diets

Eight ruminally cannulated crossbreed steers (Hereford x Angus, 501.9 ± 29.6 kg) were assigned randomly to two 4 x 4 Latin squares at the nutrition physiology barn (Oklahoma State University). Steers were weighed at initiation and completion of each 18-d period. Steers were housed in individual indoor 3 x 4-m pens for 9 d each period. On d 10, they were moved to individual metabolism stalls for a 3-d adaptation and a 6-d collection period. The treatments included: chopped alfalfa hay ad libitum and a mineral vitamin premix (CONT), chopped alfalfa hay plus 0.5% of body weight (BW) cracked corn every day (24), chopped alfalfa hay plus 1.0% BW cracked corn every second day (48), or chopped alfalfa hay plus 1.5% BW as cracked corn every third day (72). Animals were fed each morning at 0800 and had continuous access to chopped alfalfa hay and water. Supplemental corn was administered as shown in table 2.

Sample Collection and Preparation.

Intakes of hay and corn were recorded daily and refused hay was weighed back. Corn and hay samples were composited across days and animals within periods. All feeds either hay or corn were from a single source were composited across periods at trial completion and ground to pass a 2-mm screen in a Wiley mill for determination of DM, OM, NDF, ADF and CP. Total urine and feces were collected daily during the sampling period. Feces were weighed and a subsample was dried at 65 °C and stored for latter analysis of dry matter (DM), organic matter (OM), acid detergent fiber (ADF), neutral

detergent fiber (NDF), and fecal nitrogen. Urine was acidified with HCl to stop volatilization, was weighed every 12 hours and a subsample was frozen (-10°C) for later N analyses and another one for density that was measured immediately. At 0 h of d 16, 17 and 18, before being fed, CoEDTA (200 mL; 1.2 g of Co; Uden et al., 1980) was dosed via ruminal cannula for determination liquid passage rate (Kpf). During this 3-d period, at 0, 3, 6, 9, 15 and 24 h after feeding of each day, ruminal fluid and solid samples were collected each day to determine marker concentrations from three locations in the rumen (caudal-ventral, medial-ventral, and cranial ventral) of each steer and strained through eight layers of cheesecloth. A portable combination electrode pH meter (Corning 314i pH/mV/temperature portable pH meter with an ion selective field effect transistor electrode, Corning, NY) was used to determine pH. Following pH measurement, strained ruminal fluid samples were acidified with 1 ml of 7.2 *N* H_2SO_4 and store frozen (-10°C). A 30 ml blood sample was also collected on days 16, 17 and 18 at 6 and 24 hours after feeding via tail venipuncture, using vacuum containers for serum collection with no additives. Samples were immediately placed in a refrigerator, and were centrifuged and frozen within twelve hours. All samples according they were 6d collection or 3d collection were composited for laboratory analysis according to the scheme shown in Table 2.

Laboratory Analyses

Dry matter was determined by oven drying at 105 °C for 24 h. Ash content of fecal, corn and alfalfa hay was determined by ashing at 500 °C for 6 h in a muffle furnace. Nitrogen content of the alfalfa hay, corn, feces and urine was determined by

Kjeldahl N (AOAC, 1996). Corn, alfalfa hay and fecal sample NDF (procedure A, without sodium sulfite), ADF and ADIA concentrations were determined as described by Van Soest (1991)using Ankom 200 (Ankom Technology, Fairport, NY).

Ruminal fluid samples were thawed, centrifuged (10,000 x *g*; 10 min) and subsampled for Co-EDTA, NH_3-N, and VFA determination. Subsamples for VFA analysis were composited across time and days within steer and period. Concentration of Co was determined by atomic absorption spectroscopy (Model 4000, Perkin Elmer, Norwalk, CT) with an air plus acetylene flame (Hart and Polan, 1984). Ruminal NH_3-N concentration was determined colorimetrically by enzymatic procedure (Sigma, 1995). Concentrations of VFA were determined after desproteinizing 5-ml samples of ruminal fluid with 1 ml of 25% metaphosphoric acid (Erwin et al., 1961) and centrifuging at 20,000 x *g* for 15 min. Individual VFA were separated by gas chromatography (Perkin Elmer Autosystem, 9000 series) using 8 ml/min flow rate of ultra high-purity helium as a carrier gas with 2-ethylbutyric acid as an internal standard.

Calculations

Fluid dilution rate was the slope of the natural logarithm of Co concentration regressed against time (Galyean, 1997). Ruminal pH area below a line of 6.2 and pH curve and the time that pH remained below 6.2 was calculated by using spline curves (piece wise polynomial functions) with MATLAB (MathWorks Inc.). Area and time were calculated for 6 days by using the 3d curves of CONT, 24 and 72 and multiplying by 2 and for treatment 48 as shown in table 2 and multiply by 3. Apparent total tract hay OM

digestibility was calculated assuming a constant indigestibility of corn using 100 minus the tabular value of corn from the NRC (1996) .

Experiment 2. Performance Trial

Animals and Diets.

Sixty Holstein heifers (199 kg; 14 mo old) and eight cannulated heifers were stratified by weight and assigned to the same four treatments with the exception that hay was not chopped. Animals were fed in a drylot at INIA La Estanzuela Experiment Station (lat 34° 20° S, long 57° 41° W), Colonia, Uruguay for 110 d (20 d for adaptation and 90 d for measurements). Round alfalfa bales (330 kg) were offered ad libitum with a salt premix also ad lib. Due to a drought in the spring and summer alfalfa bales were of a quality lower than expected. Animals were sorted each morning at 0800 and those receiving supplemental corn were fed individually. Animals were weighed every 14 d with intake of corn DM being adjusted at this time.

Sample Collection Preparation and analysis.

Starting on d 60, animals were fed chromic oxide for 9 d with fecal samples being collected the final 3 d. A subsample was taken for latter analysis and composited according to Table 2. Subsamples were analyzed for NDF, ADF and Cr. Samples were analyzed with the same procedure of Trial 1. Calculations of intake were done as explained for trial 1.

Statistical Analysis

Trial 1. The two Latin squares were analyzed for intake, digestion, fecal output, mean ruminal value, mean VFA values, mean Kpf value. Period, animal, and treatment were

included as source of variation and the statistical analyses were performed using the GLM procedure of SAS (1999). Linear and quadratic effects of frequency of feeding were tested using contrast statements. Contrast comparison corn treatments versus control were also done. Analysis of the repeated measures was done using the SAS/MIXED procedure (version 8, 1999). For each response variable covariance structures of the repeated measures were selected based on model fit criteria.

Trial 2. The heifers were stratified by weight in a complete randomized block design. It was analyzed using the GLM of SAS. Contrast statements were used for the same comparisons as explained for trial 1.

Results and Discussion

Trial 1. Intake and total tract digestibility. Intake as % of BW was not affected by cracked corn supplementation but decreasing frequency of supplementation tended ($P<0.01$) to reduce intake (Table 3). Total organic matter intake was not affected by corn supplementation, but it was decreased linearly ($P<0.01$) by decreasing feeding frequency (Table 3). Supplementation lowered hay organic matter intake ($P<0.01$) and hay as percentage of intake ($P<0.01$) when compared to the control and decreased linearly ($P<0.01$) as feeding frequency decreased. However, DOMI was increased by supplementation ($P<0.05$) and decreased linearly ($P<0.05$) as feeding frequency decreased. It has been documented in a review by Horn and McCollum (1987) that supplementation of high quality forages may reduce forage intake with a more pronounced substitution for lower quality forages. In this trial the decrease in feeding frequency increased the rate of substitution and decreased not only intake of steers but also the proportion of hay in the diet producing a greater substitution of forage by the

supplement. In treatments 48 and 72 there was a decrease in the immediate day after feeding corn on total OM intake. The days heifers were fed corn the proportion of grain in the diet was higher than 20-30% which according to Ørskov (1986), higher than this proportion may depress intake to a larger extent. However, in treatment 24 although there was substitution, supplementation with corn increased total organic matter intake, and as reviewed by Minson (1990) a combination of moderate amounts of supplement added to forage increased total voluntary intake but this increment is less than the quantity of supplement that is included in the diet. A reduction in DM intake was also observed by Hart (1987) feeding sorghum silage and soybean meal supplemented with 26 or 39% of corn in alternate days than when it was supplemented every day.

NDF and ADF. Intake of ADF and NDF was greater by the CONT treatment ($P<0.01$; Table 4) mainly because the composition of the diet was only hay, while for the other treatments corn was between 18-22% of the total diet (Table 3) and there were no differences in total organic matter intake due to corn supplementation. Fecal output of NDF and ADF was decreased ($P<0.01$) by corn supplementation. Total tract ADF and NDF apparent digestibility was increased by corn supplementation ($P<.01$, $P=0.07$; respectively). Increasing feeding frequency decreased linearly ($P<0.01$) ADF intake, fecal output and digestibility and also linearly decreased ($P<0.01$) NDF intake and fecal output and decreased apparent total tract digestibility ($P<0.01$). Depressions in fiber digestibility are not severe until supplemented grain reaches either 20 to 30 % or 300 g kg^{-1} of intake whereas smaller inclusions may increase fiber digestion (Galyean and Goetsch, 1993). The proportion proposed by these authors for affecting fiber digestion is higher than the average of supplemented corn for the treatments in this trial, although the

same day's corn was supplemented in the treatments 48 and 72 that proportion was higher. Elizalde et al. (1999) found that fiber digestibility of fresh alfalfa was not depressed by increasing the level of corn from 0 to 1.2% of BW.

Nitrogen. Intake of nitrogen was greater by CONT ($P<0.05$) than for corn-supplemented treatments and it was decreased as feeding interval decreased ($P<0.01$). As total organic matter of CONT didn't differ from supplemented treatments this difference is expected since alfalfa hay has a larger concentration of nitrogen. Fecal output of nitrogen was not affected by corn supplementation but it was increased as the frequency of feeding increased ($P<0.02$). Heifers of all supplemented treatments ate all the same amount of corn (Table 3), but total OM intake was linearly decreased as feeding frequency decreased due to a decrease in hay intake. Therefore the reduction in nitrogen intake is mainly explained by this reduction in hay intake. Apparent total tract digestibility of nitrogen was not affected by corn supplementation but show a trend to increase ($P=0.10$) as supplementation interval increased. A linear decrease in total tract apparent digestibility and a linear decrease in nitrogen intake was observed by Elizalde et al.,(1999b) when increasing the amount of cracked corn with fresh alfalfa.

Ruminal parameters

Volatile fatty acids (VFA). Total VFAs were not affected either by corn supplementation or feeding frequency (Table 5). Neither acetate, propionate , isobutyrate, valerate or isovalerate was affected by corn supplementation or by feeding frequency. The acetate propionate relation (A/P) was affected by corn supplementation ($P<0.05$) as also butyrate concentration ($P<0.01$).

Ruminal pH was analyzed by two different approaches. First all the pH was aligned with the corn feeding time (Figure 1). Analyzed as repeated measurements, the CONT treatment was different from 24 (P=0.09) and 48 and 72 (P<0.01) and 24, 48 and 72 were different among them (P<0.01) for the first 24 hours. The second approach through polynomial functions calculated for each individual animal (Table 5) showed that the area below a line of 6.2 and the individual curve of pH and the time in hours under 6.2 was different (P<0.01) for supplemented treatments. This is in accordance with most of the literature published that show that the inclusion of grains to forage reduces ruminal pH. Increasing feeding frequency decreased in a quadratic form (P<0.05) the area below 6.2 and pH curve and decreased linearly (P<0.01) the time in hours that pH is below 6.2 (Figure 2, Table 5). A rumen pH below 6.2 will affect cellulolytic bacteria growth (Ørskov, 1986) and may affect fiber digestion in the rumen. Although the hours under 6.2 and the area below was greater for supplemented treatments and was increased by decreasing feeding frequency, NDF and ADF digestion was improved by decreasing the feeding frequency.

Dilution rate (%/h) or Kpf was not different between supplemented treatments and the control. However, there was a linear (P<0.01) decrease in Kpf as feeding frequency increased. Ruminal fluid is very important since it is the biological active and soluble fraction (Owens and Goetsch, 1993). A higher retention time in the rumen may have increased fiber digestibility explaining the results observed. Also a higher retention time will reduce feed intake as this reduction was observed as frequency decreased was observed in this trial. Stensig and Robinson (1997) found that by increasing the amount

of concentrate in the diets of dairy cows fed alfalfa silage ruminal retention time of fiber was increased, however it didn't affect intake.

Ruminal ammonia was decreased immediately after feeding time being more severe when corn was fed ($P<0.05$) and as feeding frequency increased (Figure 3). Ammonia in the rumen is available from three major sources that are the forage, the supplement and through recycle through the wall either by diffusion through the ruminal wall or via saliva (Owens et al., 1991). For these same authors with forages with more than 10 % CP, ammonia supply from the forage alone will be enough unless rumen degradable protein falls below 55% which is not the case in this trial. The amount of ammonia in the rumen reflects solubility and fermentability of the dietary and endogenous sources of N (Huntington and Archibeque, 1999). The optimum levels varies according to diet, however, values between 5 and 8.5 NH_3 mg/dl are considered in most cases enough for a normal rumen function (Roffler and Satter, 1975; Satter and Slyter, 1974). In our trial the level of ruminal ammonia was above 5 so we didn't find a biological value to study the area under the curve. Ruminal nitrogen was also decreased when increasing levels of cracked corn were supplemented (Elizalde et al., 1999b).

Blood parameters

Serum urea nitrogen show the same trends as ruminal ammonia and ruminal pH (Figure 4). There was a greater decrease in serum urea nitrogen in supplemented than in non supplemented cattle and this decrement was larger as feeding frequency increased. The principal factors affecting the rate of endogenous urea transfer from the blood to the lumen of the gastrointestinal tract are the organic matter digestibility, plasma concentration of urea, and ruminal ammonia concentration (Kennedy and Milligan, 1980)

and capillary flow and CO_2 tension (Huntington and Archibeque, 1999). Although recycling was not measured in this trial, lower pH and ruminal ammonia observed as feeding frequency decreased may be indicative of a higher fermentability the day corn was fed and a decrease in these parameters will to some extend increase the removal of urea nitrogen from blood explaining the differences observed in this trial.

Trial 2.

There were differences ($P<0.01$) in ADG and final weight in the Holstein heifers fed corn (Table 6). Supplementation of medium to high-quality forages with an energy supplement usually increases ADG (Horn and McCollum III, 1987). ADG increased ($P<0.01$) as feeding frequency increased. There are no reports that we are aware that have examined the effect of supplement feeding frequency in medium to high quality hay. These results are in agreement with what was measured in Experiment 1. Differences may be given mainly by the intake of DOMI.

These two trials suggest that supplementing alfalfa hay with corn grain increased intake of digestible OM and increased ADG. However, supplementing at less frequent intervals (48h; 72h) increased hay digestibility slightly, but reduced intake of hay OM markedly and thereby reduced intake of digestible OM and ADG

Implications

By supplementing corn it is possible to increased ADG, however, the frequency of supplementation will be a balance between the price of alfalfa, corn and labor. Supplementing every 48 h may be a reasonable option where labor and alfalfa costs are high although some ADG will be sacrifice.

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Table 1. Chemical composition of the diet (DM basis)

	Trial 1		Trial 2	
ITEM	Alfalfa	Corn	Alfalfa	Corn
DM	89.01	88.30	88.23	89.27
Ash	8.22	1.35	12.28	3.67
Nitrogen	3.36	1.40	2.53	1.42
NDF	52.30	24.93	64.91	17.04
ADF	36.03	5.23	41.22	5.78

Table 2. Scheme of feeding cracked corn supplement and how samples were composited for analysis the treatments during collection period

	Day of collection period					
	1	2	3	4	5	6
Corn as %BW						
CONT	--	--	--	--	--	--
24	0.5	0.5	0.5	0.5	0.5	0.5
48	0	1.0	0	1.0	0	1.0
72	1.5	0	0	1.5	0	0
6-d collection						
CONT	1	1	1	1	1	1
24	1	1	1	1	1	1
48	1	2	1	2	1	2
72	1	2	3	1	2	3
3-d collection						
CONT				1	1	1
24				1	1	1
48				1	2	1
72				1	2	3

Same number within row was composited for lab analysis and final statistics

Table 3. Intake, digestibility and digestible intake of organic matter (DOMI) by steers fed alfalfa hay ad libitum and three different feeding frequencies of cracked corn

	Treatment[a]					Contrasts[b]		
		Feeding Frequency (hours)						
	CONT	24	48	72	SEM[c]	CONT vs Corn	L	Q
						P		
Intake as % BW	2.57	2.80	2.50	2.34	0.09	0.81	<0.01	0.79
Intake OM kg/d								
Hay	12.64	11.49	10.09	9.47	0.48	<0.01	<0.01	0.58
Corn	---	2.48	2.48	2.46	---	---	---	---
Total	12.64	13.95	12.58	11.99	0.48	0.70	<0.01	0.55
Hay as % intake	100.00	82.21	80.04	78.51	0.73	<0.01	<0.01	0.86
Digestibility OM	68.89	71.66	73.90	75.04	1.06	<0.01	<0.01	0.64
DOMI kg/d	8.72	10.01	9.27	8.97	0.33	<0.05	0.02	0.65

[a]CONT = Control alfalfa ad libitum; 24, 48 and 72 = alfalfa ad libitum and cracked corn supplementation every day, every other day or every third day at .5, 1 and 1.5% of BW respectively.

[b]Preplanned contrasts with *P*-values control vs corn supplemented steers; L = linear Q = quadratic for corn supplemented steers only.

[c]Standard error of the mean.

Table 4. Total intake, fecal output and total tract apparent digestibility of ADF ash free and NDF ash free and nitrogen by steers fed alfalfa hay ad libitum and three different feeding frequencies of cracked corn

	Treatment[a]					Contrasts[b]		
		Feeding Frequency				CONT vs		
	CONT	24	48	72	SEM[c]	Corn	L	Q
						P		
ADF								
Intake kg/d	4.55	4.25	3.75	3.52	0.17	<0.01	<0.01	0.57
Fecal Output kg/d	2.16	1.99	1.57	1.40	0.10	<0.01	<0.01	0.39
Digestibility %	52.55	53.16	58.26	60.70	1.41	<0.01	<0.01	0.45
NDF								
Intake kg/d	6.65	6.32	5.59	5.26	0.25	<0.01	<0.01	0.57
Fecal Output kg/d	2.45	2.35	1.87	1.69	0.12	<0.01	<0.01	0.36
Digestibility %	63.15	62.79	66.61	67.90	1.30	0.07	<0.01	0.45
Nitrogen								
Intake g/d	445.83	443.21	394.39	372.89	16.85	0.03	<0.01	0.56
Fecal Output g/d	110.12	116.77	103.02	92.17	6.33	0.37	0.01	0.98
Digestibility %	75.28	73.62	74.02	75.41	0.77	0.27	0.10	0.61

[a]CONT = Control alfalfa ad libitum; 24, 48 and 72 = alfalfa ad libitum and cracked corn supplementation every day, every other day or every third day at .5, 1 and 1.5% of BW respectively.

[b]Preplanned contrasts with *P*-values control vs corn supplemented steers; L = linear Q = quadratic for corn supplemented steers only.

[c]Standard error of the mean.

Table 5. Ruminal VFA concentration, acetate to propionate ratio, dilution rate, pH area below a 6.2 line and the pH curve in 6 d and time in hours that pH was under 6.2 for 6 d of steers fed alfalfa hay ad libitum and three different feeding frequencies of cracked corn

	Treatment[a]					Contrasts[b]		
		Feeding Frequency				CONT Vs		
	CONT	24	48	72	SEM[c]	Corn	L	Q
						P		
VFA, mmol/L								
Acetate	84.67	81.78	82.26	80.01	2.49	0.22	0.60	0.63
Propionate	18.59	19.16	19.62	19.59	0.82	0.33	0.75	0.83
Butyrate	7.63	8.97	9.15	10.64	0.61	<0.01	0.06	0.17
Isobutyrate	1.87	1.88	1.83	1.85	0.06	0.77	0.71	0.67
Valerate	2.20	2.06	2.03	2.20	0.92	0.31	0.32	0.37
Isovalerate	1.91	2.04	2.04	2.14	0.11	0.18	0.51	0.69
Total	118.87	115.18	116.93	116.41	3.26	0.89	0.90	0.83
Acetate/Propionate	4.54	4.37	4.24	4.11	0.13	0.04	0.31	0.98
Dilution rate %/h	8.45	8.97	7.39	6.92	0.52	0.22	<0.01	0.36
Area pH (6d)	1.05	2.88	6.55	17.63	1.14	<0.01	<0.01	0.03
Time h (6d)	13.77	27.54	40.86	50.63	5.10	<0.01	<0.01	0.74

[a]CONT = Control alfalfa ad libitum; 24, 48 and 72 = alfalfa ad libitum and cracked corn supplementation every day, every other day or every third day at .5, 1 and 1.5% of BW respectively.

[b]Preplanned contrasts with *P*-values control vs corn supplemented steers; L = linear Q = quadratic for corn supplemented steers only.

[c]Standard error of the mean.

Table 6. Initial, final weight and average daily gain (ADG) of steers fed alfalfa hay ad libitum and three different feeding frequencies of cracked corn after 90d (Trial 2)

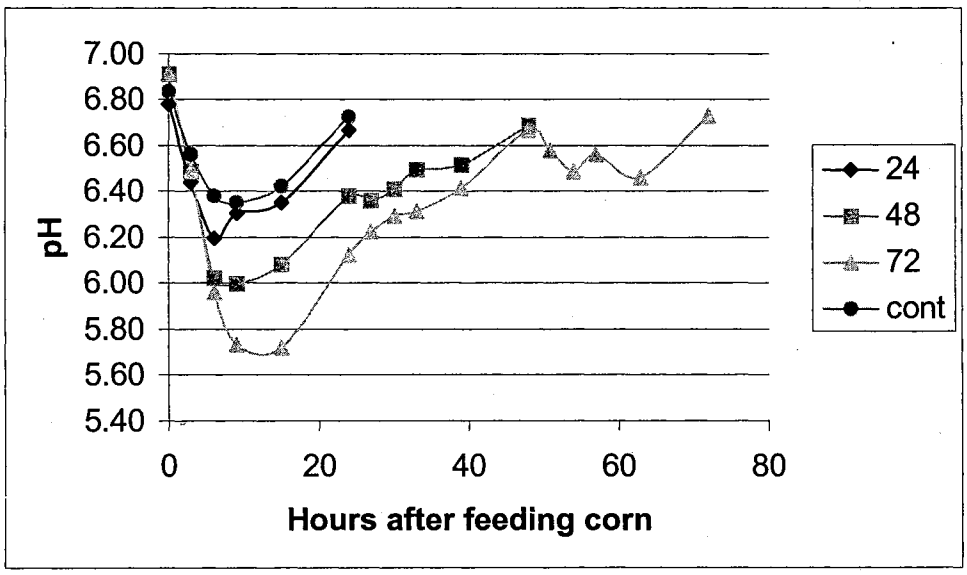
	Treatment[a]					Contrasts[b]		
		Feeding Frequency (hours)				CONT vs		
	CONT	24	48	72	SEM[c]	Corn	L	Q
						P		
Weight (kg)								
Initial	199.9	198.4	194.1	198.9	2.2	0.26	0.88	0.23
Final	243.9	267.9	261.3	254.6	3.9	<0.01	<0.01	0.98
ADG (kg)	0.48	0.77	0.75	0.62	0.03	<0.01	<0.01	0.21

[a]CONT = Control alfalfa ad libitum; 24, 48 and 72 = alfalfa ad libitum and cracked corn supplementation every day, every other day or every third day at .5, 1 and 1.5% of BW respectively.

[b]Preplanned contrasts with *P*-values control vs corn supplemented steers; L = linear Q = quadratic for corn supplemented steers only.

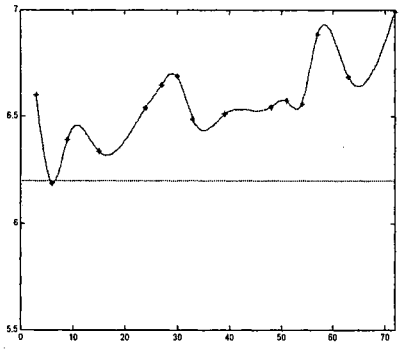
[c]Standard error of the mean.

Figure 1. Ruminal pH in steers fed alfalfa hay ad libitum and three different feeding frequencies of cracked corn

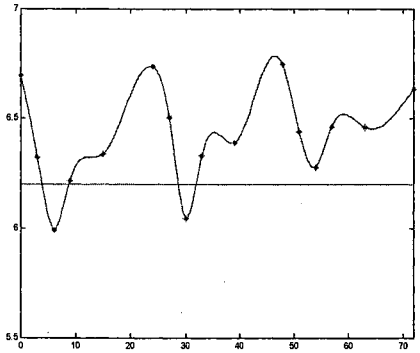


CONT = Control alfalfa ad libitum; 24, 48 and 72 = alfalfa ad libitum and cracked corn supplementation every day, every other day or every third day at .5, 1 and 1.5% of BW respectively.

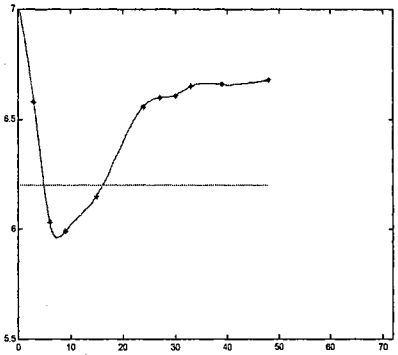
Figure 2. Example of the spline curves for the same steer in each period and treatment.



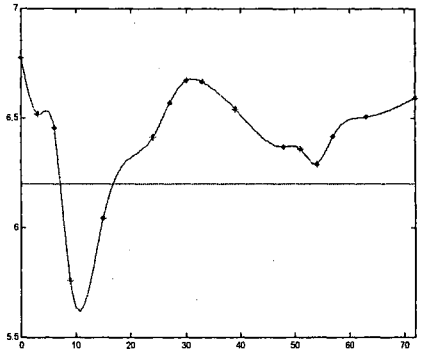
CONT



24

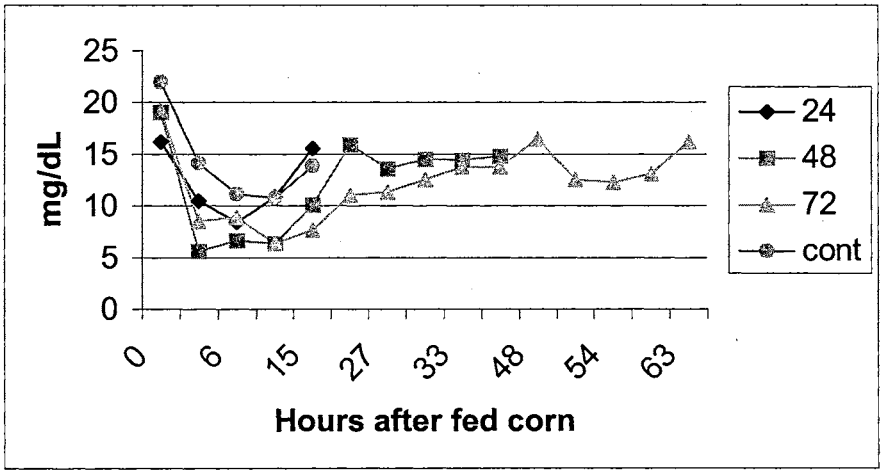


48



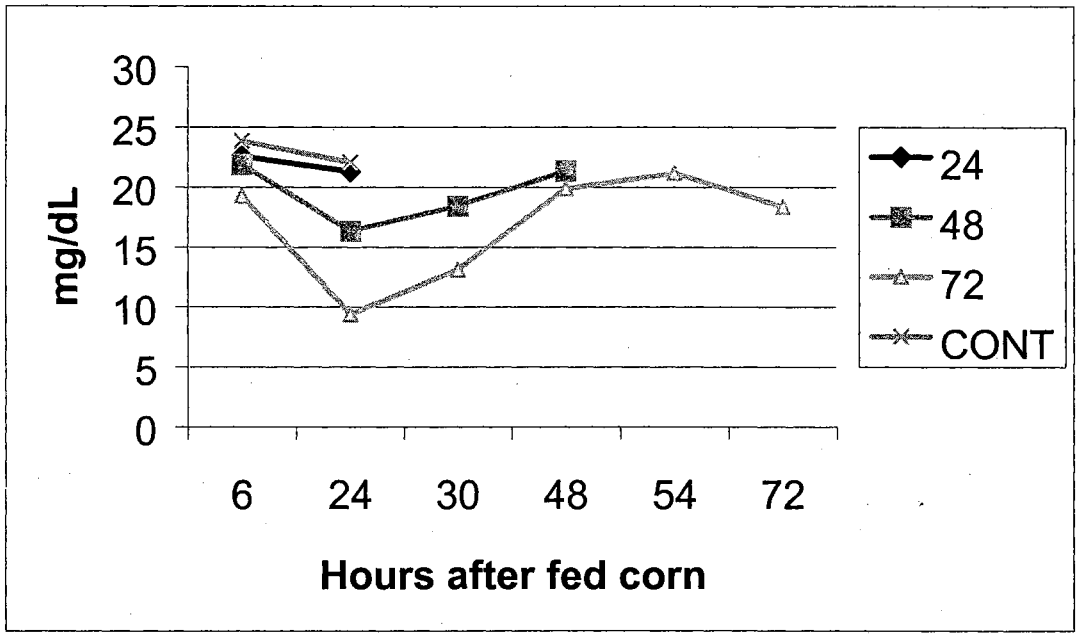
72

Figure 3. Ruminal ammonia in steers fed alfalfa hay ad libitum and three different feeding frequencies of cracked corn.



CONT = Control alfalfa ad libitum; 24, 48 and 72 = alfalfa ad libitum and cracked corn supplementation every day, every other day or every third day at .5, 1 and 1.5% of BW respectively

Figure 4. Serum urea nitrogen in steers fed alfalfa hay ad libitum and three different feeding frequencies of cracked corn.



CONT = Control alfalfa ad libitum; 24, 48 and 72 = alfalfa ad libitum and cracked corn supplementation every day, every other day or every third day at .5, 1 and 1.5% of BW respectively

CHAPTER IV

ENVIRONMENTAL IMPACT OF FIVE DIFFERENT DAIRY SYSTEMS OF URUGUAY

Abstract

Budgets of inputs, outputs and losses of five different technological systems of Uruguay are analyzed, compared and discussed. The systems include five different models that were identified according to type of crop pasture rotation, production and use of hay and/or silage, use of concentrates, stocking rate (milking and dry cows per ha) and how much of the milking potential of the cow is really used (Duran, 2000). Model I is based on natural range, annual species (oat, wheat), but no use of hay or silage. Model II incorporates improved pastures with legumes (no crop pasture rotation), phosphate fertilizers, and decreased use of concentrates per cow. Stocking rate is increased by a 40% with respect to Model I. Model III farmers use farm planning for animal nutrition, crop-pasture rotation and reproductive performance. Model IV increases the stocking rate of Model III by 40% by doubling the use of concentrates. Model V tries to increase production relative to the genetic potential of the cows, requires more concentrate and balanced diets are used to produce more milk with an increase in the stocking rate of system IV. The first three models were identified through surveys from farmers and partial modeling while models IV and V were modeled and then validated three year each for milk production, use of silage and concentrates.

Nitrogen balance for model I was not sustainable in the long term. However, for the other models, N was in excess from 24 to 73 kg^{-1} ha^{-1} increase as stocking rate and use of concentrate increase. However, efficiency of utilization of N (output/input) increased with the intensity of producion. Phosphorus accumulates in the farm at a rate from 0.6 to 8.8 kg ha^{-1} yr^{-1}. Efficiency of use of P varied from 28 to 40% having the low-input low-output models the same efficiency as the more intense model (V). The potassium balance was negative for all the systems considered. The lack of K fertilizer use and the rich K soils have hidden this loss. Eventually potassium will become a limiting nutrient in the sustainability of dairy farms in Uruguay.

Introduction

In the last 35 years world food production has doubled but that increase has occurred in association with a 6.87 fold increase in nitrogen fertilizer use, a 3.48 fold increase in phosphorus fertilizer use, a 1.68-fold increase in the amount of irrigated cropland, and a 1.1-fold increase in land in cultivation (Tilman, 1999). The increased use of fertilizer, increased stocking rates and the increased use of concentrates has also led to an increase in potential environmental problems. However, the problems with nutrient pollution are not generally the result of mismanagement by farmers, but the result of evolving agricultural systems with no direct costs or penalties associated with environmental quality (Beegle et al., 2000).

Dairy farming is an important part of Uruguayan agriculture occupying over one million hectares and generating 229 million dollars as gross product value in 1999. In the last 20 years the Dairy Sector in Uruguay has adopted better technology due to the need

to maintain income because national and international prices had declined. During this process Uruguay changed from being a milk importer to be a net milk exporter while domestic consumption increased from 270 to 470 liters per capita. Between 50 and 60 % of the total milk that is industrialized of Uruguay's is exported (Duran, 2000).

Intensification and specialization with increased stocking rates, use of the soil, and use of concentrates have been the main processes of dairy farms in Uruguay. High use of inputs may cause farm fields to be non-point sources of pollution. Nutrient management is the internationally accepted strategy for addressing non-point farm pollution (Beegle et al., 2000). Nutrient management is a mass balance that begins with an accurate quantification of nutrients entering the livestock production system and nutrients leaving the system (Tyrell, 2001).

The objective of this study is to quantify through a nutrient mass balance for nitrogen, phosphorus and potassium the environmental impact of five dairy systems in Uruguay.

Materials and Methods

Description of the models

Taking into account five production factors, Duran (2000) identified five technological models that farmers are going through in order to intensify its production. These factors are:

1. Type of crop pasture rotation
2. Production and use of hay and/or silage
3. Use of concentrates

4. Stocking rate (Milking and dry cows per ha)
5. How much of the milking potential of the cow is really used.

These models were called:

1. **Extensive Grazing**. (Model I). This system has been used for decades and is based on natural range, annual species (oat, wheat), but no use of hay or silage.
2. **Improved Grazing**. (Model II) Incorporates improved pastures with legumes, phosphate fertilizers, and decreased use of concentrates per cow. Stocking rate is increased by 40% with respect to Model I. Although improved pastures are incorporated there are not crop pasture rotations implemented. The species that are mainly used are white clover (*Trifolium repens*), birdsfoot trefoil (*Lotus corniculatus*), red clover (*Trifolium pratense*) alfalfa (*Medicago sativa*) and tall fescue (*Festuca arundinacea*).
3. **Organized**. (Model III) In this model farmers use farm planning for animal nutrition, crop-pasture rotation and reproductive performance. The preplanned crop-pasture rotation tries to maximize the use of the soil, and includes not only legumes and the species mentioned above but also annual crops for forage (maize, sorghum, oat etc.)
4. **Controlled.** (Model IV) This model increases the stocking rate of Model III by 40% by doubling the use of concentrates.
5. **Advanced.** (Model V) Currently only 60 % of the genetic potential of the cows is used. In this model more concentrate and balanced diets are used to produce more milk.

The characteristics of each model are summarized in Table 1..

The crop pasture rotation used for models IV and V was as follow (starting the year in fall)

Year 1	Improved pasture with legumes associated with wheat
Year 2	Second year old improved pasture
Year 3	Third year old improved pasture
Year 4	Fourth year old improved pasture / sorghum with chicory and red clover
Year 5	Chicory and red clover / corn and oat
Year 6	Oat / corn for silage

A 10 % percent of the area is range.

A summary of each system is presented in Table 1. Models I through III have been developed from farms, partial modeling and by surveys. Models IV and V were developed and implemented (prototyping) in practice three years (92-94 and 95-97 respectively) at La Estanzuela Experiment Station (lat 34° 20° S long 57° 41° W) Colonia, Uruguay. Because the prototypes cannot be analyzed statistically in conventional ways monitoring and disciplinary research are used to analyze the systems in detail.

The approach was to use one compartment model analysis were the inputs, outputs, and losses were as follows:

Inputs	Outputs	Losses
Imported feeds	Milk	Leaching
Imported fertilizer	Animals	Runoff
Legume fixation		Volatilization
		Denitrification

The system is represented mathematically for each nutrient as

$$dN/dt = \text{Inputs} - \text{Output} - \text{Losses} \quad (1)$$

$$dP/dt = \text{Inputs} - \text{Output} - \text{Losses} \quad (2)$$

$$dK/dt = \text{Inputs} - \text{Output} - \text{Losses} \quad (3)$$

Where the change in nitrogen (dN/dt), phosphorus (dP/dt) and potassium (dK/dt) balances are a function of the inputs, outputs and losses.

Inputs

Dry matter production for each pasture was taken from Leborgne (1980) , Duran (1992) and from the data collected monitoring pasture yields at the Research Station. Percentages of clover and legumes in the pastures were taken from the paddocks for the different years and type of pastures average. For models IV and V possible yield and dry matter production of crops and pastures, composition of the pastures, use of fertilizer, feed introduced and used in the farm, slurry composition and soil chemical properties were monitored.

Nitrogen (N) fixation from legumes was assumed to be 1 kg of N for every 30 kg of dry matter produced by the legume as it was calculated through a method with isotopic nitrogen ^{15}N for Uruguayan conditions and different legumes (Garcia et al., 1994).

Fertilizer inputs were based on the recommendations usually used for models I through III. Fertilizer inputs for models IV and V are the average of the rates of fertilizer used during the trial period.

Feed inputs from outside the farm are mainly grains and feed by products. The total amounts were described by Duran (2000). For models I and II wheat bran was used as the main feed concentrate. For models III and IV wheat bran and grains as corn and sorghum were used in a proportion of 2:1. In model V a complete ration with at least 1.8 Mcal. of net energy of lactation (Mcal NE_l/kg DM) and 16.5 % crude protein and wheat bran was used in a proportion 82:18. Wheat bran in model V was mainly used for dry cows or cows in late lactation. For heifers and calves wheat bran is the main concentrate used although the amounts differ among the models according to the stocking rate and how intensively managed it is (Mieres, com pers). No roughage is considered to come from outside the farm.

Outputs

Milk production per hectare is reported by Duran (2000) (Table 1). A 3.1 % of crude protein in milk is used for every model, which corresponds to the average value monitored. Phosphorus values in milk data from the literature vary from 0.085 % (Wu and Satter, 2000) to 0.1 % (Flynn and Powers, 1985; NRAES, 1995). For this study 0.1 % of phosphorus in milk will be used since its near partial values that have been quantified in some mineral monitoring in milk in Uruguay (unpublished data). The percentage for potassium in milk is quite constant for several different environments (Sasser et al., 1966) and 0.15 % of K in milk is used.

Cows and calves that are sold are also the nutrient outputs of each of the systems. Replacement was calculated the same for all the models and is 25 % of the adult stock. Because each system has a different calving rate and stock composition (Table 1) animals sold per hectare are shown in Table 2.

The composition of N, P and K as percentage of empty body weight for the culled cows were 2.53, 0.72 and 0.19 respectively (ARC, 1980; NRAES, 1995). For young stock the body composition in percentage of N, P and K used was 2.88, 0.83 and 0.22 % respectively (ARC, 1980). The weights at which animals are sold were 500 kg for model I and II and 570 kg for models III, IV and V. All male calves and females in excess of those needed for replacement were calculated as sold 10 days after birth at an average weight of 40 kg. No hay, silage or grain is considered to be sold from the farm.

Losses

Under Uruguayan conditions animals spend most of their time grazing so most of the urine and the manure are left in the paddocks. Although it is not considered here there are expected differences among paddocks in the different models. It is considered that the more intensive models (III to V) had a better distribution since the management is tighter and animals spend less time in each paddock and grazing strips are more controlled.

To quantify the type fecal and urinary excretion, each model was simulated and calculated using the Cornell Net Carbohydrate and Protein System (CNCPS 4.0.0.31;Cornell University, NY), with the tabulated values that appear in the Uruguayan nutritional guide for ruminants (Cozzolino et al., 1994) and when available, feed analysis done in the nutrition laboratory at La Estanzuela Experiment Station. The CNCPS

software was selected since it has been validated for type of pastures and situations similar to that of Uruguay (Kolver et al., 1996). For each model a different simulation was done for each type of animal (milking cow, dry, heifers and calves) and for three different seasons (fall-winter, spring and summer) and afterwards the annual excretion was calculated by addition. Results of N, P and K major routes of excretions and total amounts are shown in Table 3, 4 and 5 respectively.

Ammonia N losses by volatilization from urine may vary from 15 to 25% (Haynes and Williams, 1993). We used the value of 22%, which is the value that Ryden et al (1987) measured at a mean air temperature of 16 °C. For N losses in dung through ammonia volatilization the same authors measured almost 1 % and it is the value used.

Some measurements by Malcuori et al. (1999) show that 8 to 12 % of the total manure is collected in the milking parlor. However, management in the milking parlor is very important and animal excretions may vary according to the treatment animals receive. Animal nervousness, failure to keep the daily routine, and management may vary manure and urine outputs in the milking parlor. For this study we will use 12%. In models I and II no recycling to paddocks is done and usually dairy waste from the milking parlor is lost to the field, therefore, it will be taken into account as nutrient loss. The reason for this is during the time these models were developed there was no legal concern and the investment in manure recovery systems was too high for the farmer. The use of dairy waste with a high carbon (C) to nitrogen ratio (30:1) which was incorporated into the soil just before a winter crop (oat, barley or wheat) has decreased dry matter yields significantly (La Manna et al., 2001) and therefore for models III, IV and V it is calculated that excretions from the milking parlor are stored in a lagoon for later

application during spring, summer and early fall. The estimated N losses by volatilization from lagoons range from 40 to 70 % so we used a value of 60% . No losses of K and P are assumed by storage for models III, IV and V. However, since animals are moved from and back to the paddocks to the milking parlor 2 times a day each usually through a cattle trail that is not used for other purposes it was assumed the cattle spent 1 hour per day there. Assuming a fecal and urinary distribution through out the day is possible to estimate this loss in 4 % of the excretions of the dairy cows.

Application of manure to the soil without immediate incorporation can lead to losses of up to 40% of the N applied. This is the amount we assume to be lost in application to the field.

Losses from the field in Uruguay have not been divided between leaching, and denitrification, however, it is estimated that a typical N loss under normal annual grazing crops is 60 kg N ha per crop (Sawchik, personal communication). In the case of crop pasture rotations with a 50% of the time under pasture Diaz Rosello (1992) measured an average loss of 20.5 to 23.6 kg N ha^{-1} yr^{-1} with a N fertilization of 16.7 kg N ha^{-1} yr^{-1} in 36-yr trial. For P losses by erosion in the same crop pasture production systems Moron and Kiehl (1992) measured losses of 7.5 kg and of 11.46 P kg ha^{-1} yr^{-1} for crops alone with fertilization (case of model I). However, the losses measured by Diaz Rosello (1992) and Moron and Kiehl (1992) where under conditions of soil tillage and maximum erosion. For this study 70% of the values proposed above for the type of pastures will be used. These losses are reported as soil and tillage since proper identification of what type of loss is not possible.

Losses of potassium beyond the root zone in soils from urine can range from 0 to 46% of the total urine depending more on physical conditions of the soil rather than the soil chemical, mineral characteristics and moisture content (Williams et al., 1990). This type of loss called preferential loss is assumed in this work to be a 5% of the potassium in the urine according to the type of soils (clay with an impermeable B horizon) where the trial was carried out.

Efficiency of nutrient use was calculated as amount of nutrients in the output divided by input of the same nutrient multiplied by 100. Surplus was calculated by the subtraction of inputs minus outputs for each nutrient.

Results and Discussion

Nitrogen. Inputs increased in all the farms according to the intensity of production (Table 6). Except for model I, which does not use legumes as do the other models, legume N, is an important percentage of the total inputs (74.5, 77.9, 67 and 60 for models II, III, IV and V). This is logical in grasslands systems that rely in legumes like the ones found in Argentina, New Zealand and Uruguay. In other systems where the prices of feed and fertilizer are lower, feed is the most important input of N to the farm (Klausner, 1995). Under Uruguayan economic conditions legume N is of great importance achieving at low cost production and therefore have a competitive product for export. As stocking rate increases, the concentrates that are used for cows increase in importance. In model V, feed for the cows is the second major input while for the other models fertilizer is the second major input. The more important N output is milk for all the models since there is

not grain, hay or silage. Milk output is the major N output in most of the NY dairies also (Bouldin and Klausner, 1998).

All the models except model I show a low utilization of nitrogen of between 16.9 to 22.2 % (Table 6). Model I is a low input-output system and is not profitable under the actual economic conditions of Uruguay. Also from a nitrogen stand point model I is not sustainable. For this model to be sustainable improved pastures with legumes should be included since only N fertilizer doesn't improve soil N in the long run (Whitehead, 1995), while improved pastures increases soil OM and therefore increases soil total N (Diaz, 1992). There is an increment in nitrogen efficiency of utilization from Model II through V mainly explained for a higher milk production and a higher number of animals that are culled. These two factors, milk produced and animals culled, are the result of better management and a more balanced feeding for dairy cows which increases efficiency per hectare (Table 6). The increase in stocking rate allows a better utilization of the grass and an even distribution of urine and feces. In this modeling the assumption is that the increase in DM per hectare is not so much because of yield increment but rather for better forage utilization and more hectares under improved pastures. This has been shown in several trials and our monitoring of the prototype systems in the Dairy Unit. The monitoring results of models IV and V resulted in an estimated 75 and 82% of forage utilization, respectively, which is high for Uruguay.

Typical farms in the United Kingdom show total N inputs between 300-400 kg N ha^{-1} with offtakes of 60-80 kg N ha^{-1} (Jarvis et al., 1996; Peel et al., 1997) which is to a similar efficiency as the models showed above but with a greater surplus per ha. Not all the excess is lost to the environment, N may accumulate between 30 to100 kg ha^{-1} yr^{-1} in

the N soil pool (Aarts et al., 1992; Berentsen and Giesen, 1995). However, in the long run this accumulation may led to more leaching losses of N into groundwater (Goh and Williams, 1999).

Phosphorus. The first two models I and II are low inputs low outputs. The efficiency of utilization of P inputs is 40 and 33 % respectively (Table 7). Analyzing Models III to V there is an increase in P efficiency mainly due to high stocking rate, which allows better grass utilization and higher milk production. Reproductive performance also explains in part this improvement in efficiency since more animals are sold for outside the farm. Despite the fact that Model V has a greater stocking rate per ha, the use of a more balanced feed intake increases the efficiency of P utilization and at the same time, the net income per ha in more than doubled.

Similar models for dairy farms in New Zealand, England and the Netherlands resulted in a P efficiency of 17.8, 38.2 and 33.3 % respectively and the total P that stayed in the farm was 49, 26.7 and 32 kg P ha^{-1} yr^{-1} (Goh and Williams, 1999). The net gain of P in the soils of all the models eventually will increase until the possibility of runoff and soil erosion increases and therefore, the possibility of eutrophication of waterways increases. In Uruguay levels of P in soils are from 3 to 20 ppm. Most of the soils have a low P fertility so its not a problem.

Potassium. Potassium shows a deficiency for all the models considered. Although nutrient efficiency for models I and IV are 45 and 64% which is given by a low input-output for model I and a high use of wheat bran per ha as imported feed for model IV,

final balance is stillnegative. Farmers in Uruguay are not used to utilize K as a fertilizer and the soils have high levels of K that dissimulate the losses. Eventually potassium in the long run will become a key component for sustainability. The cost of being sustainable in the future for all the models will depend on close monitored of soil status, even distribution of the waste in the different paddocks and the use of potassium fertilizer.

In Dutch farms there is imported around 108 kg of K ha^{-1} yr^{-1} and 30 kg of K ha^{-1} yr^{-1} of feed with a utilization from 10 to 30% (Aarts et al., 1992). Similar utilizations of k were observed in dairy farms of New York (Bouldin and Klausner, 1998).

Implications

The five models studied show the big trends of nitrogen, phosphorus and potassium in the farm for five models that differ in intensity of stocking rate, supplementation, and soil and forage utilization in Uruguay. For N and P there is a surplus that increases as intensity increases. However, the efficiency of use also increases with intensity. Potassium on the other hand is deficient and may be a key nutrient for future sustainability.

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Table 1. Principal technical parameters for the 5 models

Models:	I	II	III	IV	V
ROTATION	no	no	yes	yes	yes
Improved Pastures with legumes (% of total area)	0	50	65	60	60
Dry Matter /ha	very low	medium	high	max	máx
Use of Hay	very low	high	low	very low	none
Concentrate(kg/cow)	660	420	670	1200	1600
" (kg/ha)	231	252	469	1200	1712
Stocking rate (animals /ha)					
Cows	0.3	0.5	0.7	1.0	1.07
Heifers 2-3	0.075	0.125			
Heifers 1-2	0.075	0.125	0.175	0.25	0.268
Calves	0.075	0.125	0.175	0.25	0.268
Milk (liters/cow)	2200	3800	4500	4700	6315
" (liters/ha)	760	2000	3100	4700	6700
Calving Season	continuous	variable	fall 50%	fall 50 %	fall 100%
Mating	bull	bull	bull / A.I	A.I.	A.I
Calving interval(months)	18	16	14	13	13
First mating (age)	36	18-24	18-24	18	15
COST (U$S/ lt)	--	0.130	0.126	0.121	0.110
Net Income U$S/Ha (Liter at 0.18 U$S)		100	167	277	469
" " " (liter at 0.14 U$S)		20	43	89	201

AI: artificial insemination; lt: liters; ha: hectares

Adapted from Duran 2000

Table 2. Annual sale of animals per hectare for the different models

Model	I	II	III	IV	V
Cows (culled)/ha	0.08	0.13	0.18	0.25	0.27
Calves/ha	0.12	0.25	0.43	0.67	0.72

Table 3. Total excretion of Nitrogen and route of excretion for all the animals in the five models

Models	I	II	III	IV	V
N Excretion kg $ha^{-1}yr^{-1}$					
Dairy Cows					
Urine	5.76	10.07	15.91	31.47	33.54
Feces	7.90	15.39	24.97	48.32	43.51
Total	13.66	25.46	40.87	79.77	77.02
Dry Cows					
Urine	1.98	2.78	3.45	3.50	3.90
Feces	5.69	6.60	9.67	9.73	10.10
Total	7.67	9.38	13.13	13.23	14.00
Heifers and Calves					
Urine	2.56	3.44	4.11	8.16	8.32
Feces	5.91	8.09	9.07	14.96	14.57
Total	8.48	11.52	13.18	23.11	22.90
Total N excretion kg $ha^{-1}yr^{-1}$					
Urine	10.30	16.29	23.47	43.13	45.76
Feces	19.50	30.08	43.71	73.01	68.18
Total	29.80	46.36	67.18	116.14	113.94

Table 4. Total excretion of phosphorus and route of excretion for all the animals in the five models

Models	I	II	III	IV	V
P Excretion kg $ha^{-1}yr^{-1}$					
Dairy Cows					
Urine	0.06	0.12	0.19	0.32	0.32
Feces	1.74	4.03	6.21	12.91	11.44
Total	1.80	4.14	6.39	13.23	11.77
Dry Cows					
Urine	0.05	0.05	0.09	0.10	0.10
Feces	0.92	0.90	1.52	2.61	2.24
Total	0.97	0.94	1.61	2.71	2.34
Heifers and Calves					
Urine	0.04	0.05	0.06	0.11	0.11
Feces	0.59	0.99	1.13	2.95	3.54
Total	0.63	1.06	1.19	3.06	3.66
Total P excretion kg $ha^{-1}yr^{-1}$					
Urine	0.15	0.22	0.34	0.53	0.53
Feces	3.25	5.92	8.86	18.47	17.22
Total	3.40	6.14	9.20	19.00	17.75

Table 5. Total excretion of potassium and route of excretion for all the animals in the five models

Models	I	II	III	IV	V
K Excretion kg $ha^{-1}yr^{-1}$					
Dairy Cows					
Urine	10.34	21.85	31.51	60.60	40.10
Feces	4.16	8.41	12.74	23.73	19.77
Total	14.50	30.26	44.25	84.32	59.88
Dry Cows					
Urine	6.49	8.21	11.74	10.47	10.86
Feces	2.66	3.12	4.93	4.64	4.91
Total	9.15	11.33	16.68	15.11	15.84
Heifers and Calves					
Urine	7.23	9.43	7.65	19.40	20.08
Feces	2.73	3.69	2.79	7.09	3.56
Total	9.95	13.12	10.44	26.49	27.43
Total K excretion kg ha^{-1} yr^{-1}					
Urine	24.06	39.49	50.90	90.47	71.04
Feces	9.55	15.22	20.46	35.46	28.24
Total	33.61	54.71	71.36	125.93	99.28

Table 6. Mass balance for N for the five models (kg N ha^{-1} yr^{-1})

	I	II	III	IV	V
Inputs kg N ha^{-1} yr^{-1}					
Feed Cows	4.88	5.32	8.72	22.53	38.58
Feed Heifers	0.04	0.14	0.39	1.28	1.81
Fertilizer	2.75	11.55	14.38	25.71	25.71
Legume Fixed	0.00	49.74	82.68	101.91	101.91
Total Inputs	**7.67**	**66.75**	**106.16**	**151.43**	**168.02**
Outputs kg N ha^{-1} yr^{-1}					
Milk	3.69	9.40	15.06	22.84	32.55
Culled Cows	0.95	1.58	2.52	3.61	3.86
Culled Calves	0.14	0.29	0.49	0.77	0.83
Total outputs	**4.78**	**11.27**	**18.08**	**27.21**	**37.24**
Inputs – Outputs	**2.89**	**55.48**	**88.08**	**124.21**	**130.78**
Outputs/Inputs	**0.623**	**0.169**	**0.170**	**0.180**	**0.222**
Losses kg N ha^{-1} yr^{-1}					
Storage and parlor	1.64	3.06	2.94	5.74	5.55
Volatilization (excreta)	2.30	3.60	5.15	9.33	9.81
Cows trail	0.55	1.02	0.26	0.53	0.47
Tillage and soil	5.40	23.26	33.24	36.91	36.91
Total Losses	9.89	30.93	43.85	56.61	57.06
Input-outputs-losses	**-6.99**	**24.54**	**44.23**	**67.60**	**73.72**

Table 7. Mass balance for P for the five models (kg P ha^{-1} yr^{-1})

	I	II	III	IV	V
Inputs kg P ha^{-1} yr^{-1}					
Feed Cows	2.40	2.62	3.64	9.54	10.74
Feed Heifers	0.02	0.07	0.19	0.63	0.89
Fertilizer	0.87	5.06	10.05	10.15	10.15
Total Inputs	**2.69**	**7.74**	**13.88**	**20.32**	**21.78**
Outputs kg P ha^{-1} yr^{-1}					
Milk	0.76	2.00	3.10	4.70	6.70
Culled Cows	0.27	0.45	0.72	1.03	1.10
Culled Calves	0.04	0.08	0.14	0.22	0.24
Total outputs	**1.07**	**2.53**	**3.96**	**5.95**	**8.04**
Inputs – Outputs	**1.62**	**5.21**	**9.92**	**14.37**	**13.75**
Outputs/Inputs	**0.398**	**0.330**	**0.285**	**0.293**	**0.369**
Losses kg P ha^{-1} yr^{-1}					
Storage and parlor	0.22	0.50	0.00	0.00	0.00
Tillage and soil	0.72	3.48	5.01	5.01	5.01
Cows trails	0.07	0.17	0.26	0.53	0.47
Total losses	1.01	4.15	5.27	5.54	5.49
Inputs-outputs-losses	**0.61**	**1.07**	**4.65**	**8.83**	**8.26**

Table 8 Mass balance for K for the five models (kg K ha^{-1} yr^{-1})

Models	I	II	III	IV	V
Inputs kg K ha^{-1} yr^{-1}					
Feed Cows	2.68	2.93	4.15	10.88	9.84
Feed Heifers	0.02	0.07	0.19	0.70	1.00
Fertilizer	0.00	0.00	0.00	0.00	0.00
Total Inputs	**2.71**	**3.00**	**4.37**	**11.58**	**10.84**
Outputs kg K ha^{-1} yr^{-1}					
Milk	1.14	3.00	4.65	7.05	10.05
Culled Cows	0.07	0.12	0.19	0.27	0.29
Culled Calves	0.01	0.02	0.04	0.06	0.06
Total outputs	**1.22**	**3.14**	**4.88**	**7.38**	**10.40**
Inputs – Outputs	**1.48**	**-0.14**	**-0.51**	**4.20**	**-0.16**
Outputs/Inputs –	**0.45**	**-1.05**	**-1.15**	**0.64**	**-1.02**
Losses K kg ha^{-1} yr^{-1}					
Storage and parlor	1.74	3.63	0.00	0.00	0.00
Cows trail	0.58	1.21	1.77	3.37	2.40
Preferential flow[1]	1.11	1.78	2.27	3.99	3.19
Total losses kg ha^{-1} yr^{-1}	3.43	6.62	4.04	7.36	5.59
Inputs-outputs-losses	**-1.95**	**-6.76**	**-4.55**	**-3.17**	**-5.74**

[1] Preferential flow amount of K in urine below the root zone

VITA

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