CATEDRA DE ALIMENTACION
(Prof. Dr. EDUARDO ZORITA)

UTILIZATION OF UREA BY LACTATING EWES AND GROWING LAMBS*

by
Francisco Javier Ovejero

TABLE OF CONTENTS

LIST OF TABLES

---

* Este trabajo ha sido realizado en el Departamento de Producción Animal de la Universidad de Cornell (U.S.A.) y representa la Memoria presentada por el autor a la «Graduate School» de dicha Universidad como parte de los requisitos necesarios para la colación del grado de «Doctor of Philosophy» que obtuvo en junio de 1971.

LIST OF FIGURES

INTRODUCTION

The removal of the natural proteins from the rations, also eliminates an important source of mineral elements that may be needed for maximum productivity. Since the NPN compounds used instead of the protein supplements contain no mineral elements, these will have to be added to the rations to fulfill their requirements.

There is very little information in the literature on the effects of adding minerals to practical diets for ruminants in which NPN compounds substitute natural proteins supplying most of the dietary nitrogen. It is thought that some rations containing NPN could be marginal, or even deficient, in some mineral elements and that supplementing these rations with the minerals, should improve their utilization by the ruminants.

There is ample evidence in the literature that urea can efficiently replace some of the protein in the rations of lactating cows, and VIRTANEN (1968) reported yearly yields of more than 4.000 kg. of milk in cows fed purified diets in which NPN was the only source of nitrogen. However, there is very little information on the utilization of NPN compounds by lactating ewes, even though some reports indicate that urea can be safely used in the rations of pregnant and lactating ewes with single lambs. Practically no information has been found on the use of NPN compounds as the main source of nitrogen for ewes suckling twin lambs, which in general have

received very little attention by the researchers in animal nutrition. It is felt that «twin lambs» is a character that sheep producers may look for in the future and it is unrealistic to assume that ewes suckling twins can be handled and fed according to the standards and requirements set for ewes suckling single lambs.

To obtain more information on some of the problems mentioned above, this work was carried out with the following overall objectives:

1. To measure the effect of the addition of minerals to urea diets to equate them to an isonitrogenous soybean meal (SBM) diet, when fed to growing lambs at two different levels of protein.

2. To compare urea versus SBM as the main source of nitrogen in diets with different protein levels fed to ewes suckling twin lambs during the early lactation.

LITERATURE REVIEW

There is a very extensive literature dealing with nitrogen metabolism and specifically the utilization of Non Protein Nitrogen (NPN) compounds by ruminants. Reviews covering most of the general and more specific aspects of the nitrogen metabolism have been recently written by: HUNGATE (1966), WALDO (1968), MCDONALD (1968), TILLMAN and SIDHU (1969), SMITH (1969), VIRTANEN (1969) and PURSER (1970). Reviews dealing specifically with the NPN utilization by ruminants are due to: REID (1953), BRIGGS (1967), GARRIGUS (1968), LOOSLI and MCDONALD (1968), CHALUPA (1968) and OLTJEN (1969). It is felt that these reviews are a rather complete summary of the current literature dealing with the nitrogen utilization by ruminants and therefore only those articles that are of direct interest to the present study are included here.

General Mechanisms of Nitrogen Utilization in Ruminants.

Most of the nitrogenous material reaching the rumen is degraded by the rumen microbes to ammonia (MCDONALD, 1948), while some of it may escape unattacked to the lower digestive tract and follow digestion as in the case of the monogastric animals. The proteins in the rumen are first hydrolized to amino acids which in turn are deaminated to ammonia. The NPN compounds, mainly urea, either from the feed or from endogenous origin, are rapidly hydrolized to ammonia and CO_2 (HUNTANEM and GALL, 1955).

The ammonia nitrogen is utilized by the microbial population for the synthesis of their proteins, mainly through amination and transamination reactions. Glutamic dehydrogenase seems to be the most important enzymatic system involved in the fixation of ammonia into bacterial protein (CHALUPA, 1970a).

Many rumen bacteria prefer ammonia to amino acids (HUNGATE, 1966) for the synthesis of protein, and ammonia has been shown to be an essential nutrient for some of the species present in the rumen (BRYANT and ROBINSON, 1963). Ammonia seems to play a very important role in the nutrition of both cellulolytic and amylolytic bacteria. The protozoa do not use ammonia directly, but by feeding on the bacteria they can provide the host with a more digestible protein (CHALUPA, 1968).

If the rate of ammonia production exceeds that of uptake by the bacteria, the ammonia accumulates in the rumen creating a high rumen ammonia concentration. Excess ammonia in the rumen is absorbed through the rumen wall into the portal systen and from there it is taken to the liver where it is metabolized to urea and some is utilized in the synthesis of non essential amino acids. Ammonia escaping the rumen may also be absorbed in other parts of the gastrointestinal tract (MCDONALD, 1948). The absorption of ammonia from the rumen is dependent both upon its concentration and on the pH (HOGAN, 1961; VISEK, 1968).

The urea produced in the liver, passes into the general circulation and part of it is returned to the rumen while the excess is excreted in the urine. The blood urea may be recycled into the

rumen by two different mechanisms, either via the saliva (SOMMERS, 1961) or by diffusion across the rumen wall (HOUPT, 1968, 1969; SIMONET *et al.*, 1957; COCIMANO and LENG, 1968). The amount of urea taken into the rumen by diffusion across the rumen wall seems to be quantitatively more important than the one taken via saliva.

The mechanism of the transport of the urea molecule from the blood into the rumen is not known. However, HOUPT and HOUPT (1968) based on their experimental observations, hypothesized that the urea molecule passes across the rumen wall first by diffusion through the basal epithelial layers, where it is hydrolized by the action of the bacterial urease to ammonia and carbon dioxide. The ammonia molecule being smaller and more lipid soluble than that of the urea will go through the cornified layers of the rumen epithelium into the rumen at a faster rate itself. The net result will be a faster transfer of the blood urea into the rumen. VARADAY *et al.* (1967, 1969) observed that the transfer of blood urea into the rumen was dependent to some extent on the rumen ammonia concentration, which would agree with Houpt's general hypothesis. In any case this idea is far from being generally accepted and still has to be critically tested.

Levels of rumen ammonia from 0 to 130 mg./100 ml. have been reported in the literature (JOHNS, 1955). ANNISON (1956), indicates that under normal feeding conditions the rumen ammonia concentration is about 10 to 60 mg./100 ml. When the rumen ammonia reaches levels higher than 55 to 60 mM per liter (93.5 to 102.0 mg./100 ml.), the liver cannot metabolize the extra amount of ammonia and the peripheral blood ammonia level increases (LEWIS, 1958). Acute symptoms of toxicity are observed when the level of ammonia in the peripheral blood reaches values of 1 to 4 mg./100 ml. (CHALUPA, 1968).

Adaptation Response and Urea Utilization

REPP *et al.* (1955), reported that lambs, receiving diets in which urea and several other NPN compounds replaced as much as 50 percent of the protein in the diet, required a period of two to three weeks to become fully adapted to the NPN compounds. SMITH *et al.* (1960), using multiple regression analyses tried to quantify this adaptation response to urea and reported the existence of a linear relationship between the time the urea was fed and the nitrogen utilization. They reported that the retained nitrogen was increased by 0.20 percentage units each day of urea feeding up to 50 days.

LOOSLI and CAMPBELL (1961) also reported adaptation to NPN compounds in lactating cows fed natural diets. Diethylstilbestrol (DES) has been found to substantially increase nitrogen retention in growing lambs and at the same time reduces the time needed to become adapted to the NPN in the diet. WELCH *et al.* (1957) and MCLAREN *et al.* (1959), reported that the inclusion of DES reduced from 35 to 10 days the priod of adaptation to a urea containing diet measured as improvement in the nitrogen retention. DES did not affect the apparent digestibility of the nitrogen. PRESTON (1968) observed a decreased plasma urea nitrogen (PUN) after the administration of DES. More recently, GREBING *et al.* (1970) concluded that DES has a true protein anabolic effect in ruminants within three to five days of its administration. KARR *et al.* (1965) also observed this DES effect and reportet that the combination of dehydrated alfalfa and DES produced a better response in lambs when urea was the primary source of nitrogen.

MCLAREN *et al.* (1965), SCHAADT *et al.* (1966), CLIFFORD and TILLMAN (1968) and YAMOOR *et al.* (1968), also have reported some type of adaptation response in lambs fed purified or semipurified diets in which the NPN compounds were the main nitrogen source, when these rations were fed to growing lambs.

VIRTANEN (1966, 1967) reportet that after the administration of 15N urea to lactating cows being fed a purified diet with NPN compounds (urea and ammonium salts) as the only nitrogen source, the labelling of amino acids in the milk protein was stronger in the cows previously adapted to the NPN supplemented diet than in the cows that were not adapted. In experiments with purified diets, using soy protein as the source of nitrogen, OLTJEN and PUTNAM (1966) reported the existence of an adaptation response to the soy protein similar to that described for the diets containing NPN compounds.

JOHNSON and MCCLURE (1964) in studies both *in vitro* and *in vivo*, could not demosnstrate

the existence of any adaptation response to NPN compounds in mature sheep fed natural diets. CAFFREY *et al.* (1967) also working with mature animals reported that the nitrogen balance was not influenced by the time the lambs remained on the urea diets. They explained their disagreement with the previous reports as being due to the difference in the animals used in the experiments (mature versus growing). CAFFREY and SMITH (1964) found no evidence of improved nitrogen retention after infusion of ammonium chloride to lambs for periods of up to 60 days.

The mechanism of this adaptation response is not well understood and there are conflicting reports as to where this adaptation is taking place. MCLAREN *et al.* (1959, 1960) indicated that the better utilization of the urea diets with time was due to a better nitrogen utilization at the tissue level. LEWIS (1960) reported that the adaptation of sheep to high doses of ammonium salts is restricted to the microorganisms within the rumen, and apperars to be achieved within a week. However BARTH *et al.* (1961) in *in vitro* studies were not able to demonstrate any increase in protein synthesis with time. VIRTANEN (1969) reports that the protozoa in the rumen of adapted lactating cows have generally disappeared while the number of the bacteria have been substantially increased. CAFFREY *et al.* (1967) demonstrated in experiments both *in vivo* and *in vitro*, that the microbial adjustment to a high urea diet was accomplished within thirteen days. The same authors also reported that under high rumen ammonia concentrations the urease activity in the rumen is reduced. GARRIGUS (1968) goes on to suggest that this reduced urease activity over a period of time on a high urea diet could be a factor in the improved nitrogen utilization.

PAYNE and MORRIS (1969) found that the concentration of certain urea-cycle enzymes was significantly greater in sheep receiving high protein diets than in those fed a low protein diet. The same authors also reported that the administration of urea to sheep previously fed the high protein diet, did not produce any increase in blood ammonia concentration, while giving the same amount of urea to sheep previously fed a low protein diet increased blood ammonia concentration exponentially with time and the animals eventually died. CHALUPA *et al.* (1970b) reported that urea fed sheep were able to detoxify additional ammonia by a mechanism involving an increased concentration of liver ornithine, but they could not demonstrate any increase in the urea cycle enzymes.

From the results of the work reviewed in this area, it seems that there is an adaptation to high nitrogen intakes rather than to the intake of urea specifically, and that substantial changes take place both at the rumen and at the tissue level in order to handle the extra ammonia produced more efficiently. This adaptation may be more important when urea or other NPN compounds are fed instead of natural proteins and the intensity of the changes will vary with the different diets and also with the physiological status of the animal.

Carbohydrates and Urea Utilization.

It is a well established fact that nitrogen and carbohydrate metabolism cannot be studied independently one of the other and this becomes especially true when NPN compounds are used as the main source of nitrogen in the diet. The rumen microorganisms need to have available some source of carbon skeletons to attach the amino group during the process of microbial protein synthesis. It is highly desirable to have in the media (rumen) an energy source that is available at a similar rate as the ammonia that is being produced, in order to encourage a rapid microbial protein synthesis in the rumen (LEWIS and MCDONALD, 1958).

Early studies in this area showed that not all sources of carbohydrates were equally efficient in supporting bacterial growth. Studies both *in vivo* (WEGENER *et al.*, 1940; JOHNSON *et al.*, 1942; MILLS *et al.*, 1942, 1944; PIERCE, 1951; BELL *et al.*, 1953; HEAD, 1953; FONTENOT *et al.*, 1955; LEWIS and MCDONALD, 1958; ELLIS and PFANDER, 1958; MCINTYRE and WILLIAMS, 1970) and *in vitro* (PEARSON and SMITH, 1943; ARIAS *et al*, 1951; HUNT *et al.*, 1954; BELASCO, 1956) have shown that starch is superior to soluble sugars like glucose as well as to those slowly available to the rumen bacteria like cellulose. Molasses also was found to be inferior to starch. However, the addition of molasses is a rather convenient and safe way of feeding urea (LOOSLI and MCDONALD 1968) and makes the handling of the feed more convenient.

McLaren *et al.* (1965) showed that the retention of the absorbed nitrogen was significantly imrpoved by two percentages units per 100 kcal. of readily available carbohydrate in the diet. This imrpovement took place whether or not the lambs were adapted to the urea diets.

The addition of excess of readily available carbohydrate will depress the digestibility and utilization of the cellulose fraction of the diet. McLaren *et al.* (1965), reported that the digestion of crude fiber was significantly reduced by eight percentage units for each 1.000 kcal. of readily fermentable carbohydrate in the diet.

Hemsley (1964, 1966) indicated that cellulose could serve as a suitable energy substrate together with urea for microbial protein synthesis. Hemsley fed purified diets to lambs in which the cellulose made up to 72 percent of the dry matter and provided approximately 86 percent of the digestible energy. Nitrogen balance was decreased as starch and glucose were replacerd with cellulose, a reduction that is explanained as being due to a lower intake of digestible dry matter, rather than to changes in the chemical composition (Hemsley, 1966). Other factors such as lignification may have also been an important reason for the poor performance of the animals fed the low quality roughages supplemented with urea.

Virtanen (1966) reported that with milking cows fed purified diets the proportion of starch cannot be reduced very much without being followed by a decrease in milk production. The same author (Virtanen, 1969) goes on to say: «...there is no danger of too high urea rations provided that the feed contains enough carbohydrates of good digestibility.»

Chalupa *et al.* (1964, 1970a) suggested that a constant energy to nitrogen ratio is a very important factor when different sources of nitrogen are compared. Crampton (1964) proposed that the nitrogen requirements should be expressed relative to energy. A ratio of 20 gm. of digestible protein to one MegCal. of digestible energy (DE) is reported as the probable maintenance requirement for ruminants (Crampton, 1964; Preston, 1966). Preston *et al.* (1965) reported that the optimal ratio between digestible protein and DE for growing finishing lambs was 22 : 1. The optimum energy to protein ratio for maximum production in ruminants is not known at the present time. Purseer (1970) reviewing this very confused area, listed a series of ratios described in the literature that range from values as low as 2.3 mg. to values as high as 54.2 gm. digestible protein per MegCal of DE. No work on the establishment of this ratio for ruminants fed urea supplemented rations has been found.

Since the use of NPN compounds together with forages with a high cellulose content may be increased in the future feeding of ruminants, it is felt that more work is needed in establishing the optimal rations of nitrogen to energy under different feeding conditions, in order to be able to obtain the maximal utilization of the available resources.

Mineral Supplementation and Urea Uitilization

There is very little information available on the effects of the addition of minerals on the utilization of natural diets in which urea provided most of the nitrogen. Most of the work in this area has been done either in *in vitro* experiments or with the use of purified diets and in both cases the work was aimed at obtaining an adequate culture medium for the rumen microorganisms. The conclusions of this work have been very valuable and helpful in the developing of purified diets for ruminants, but it is felt that they cannot be adopted in animals fed natural diets without certain qualifications.

Early *in vitro* work (McNaught *et al.*, 1950; Burroughs *et al.*, 1951) showed that the rumen bacteria needed some mineral elements to obtain maximum growth and that the addition of phosphorus and iron besides the minerals normally added to the artificial saliva, were effective in improving urea utilization and cellulose digestion. Burroughs (1950) reported that a water extract of dehydrated alfalfa meal or its ash improved the digestibility of corncobs by steers. Gosset *eqt al.* (1956) and Oltjen *et al.* (1959) reported that the addition of trace minerals to the rations of fattening beef cattle consisting primarily of prairie hay and milo grain, did not produce any improvement in either gain or feed efficiency, however, the addition of these minerals to similar fattening rations containing corn instead of milo produced a marked increase in both gain and feed efficiency. Gosset *et al.* (1962) indicated that the addidions of trace minerals to fattening beef steers rations was of no value, but the rations used in these experiments contained alfalfa and molasses, both of them known to be fairly good mineral sources.

Thomas *et al.* (1953) and Nelson *et al.* (1957) reported that supplementation of urea diets with trace minerals produced gains in beef steers similar to those obtained with soy bean meal. Cattle grazing dried range grass were unable to utilize much of the urea adted as a supplement to provide about one third of the supplemental nitrogen, but when trace minerals were added to the supplement, the gain of the cattle was significatly increased.

Clark (1969) reported similar results with supplements of manganese, zinc, copper and iron added to roughage diets supplemented with either urea or SBM. Clark indicated that an extra amount of zinc improved daily gain and feed efficiency. Coombe and Christian (1969) reported a better utilization of urea, measured as improved nitrogen balance, when basal diets consisting of oat straw and urea were supplemented with a mineral mixture. The authors of this work claimed that phosphorus was the most important factor in the mineral mixture. Working with purified diets fed to sheep, Bunn and Matrone (1968) reported an improvement of growth in animals receiving cations (sodium and potassium bicarbonate) in the diets. The inclusion of alfalfa also improved growth. In *in vitro* studies, the same authors showed that either sodium or potassium bicarbonate was a better buffering agent than NH_4 within the pH range of four to six.

Oltjen and Davis (1963) in studies with steers, did not find any benefit from the addition of several buffers (calcium carbonate, magnesium sulphate and potassium chromate) to diets containing either urea or SBM. Feeding purified diets to sheep, Oltjen *et al.* (1962) reported that a 6.5 percent alkaine mixture together with a cellulose level of 30 percent, produced the best daily gain when fed to sheep.

The only mineral that has been shown to be needed in urea supplemented diets is sufur (S). Most of the work done on sulfur supplementation has been done in sheep and very little information is available for other rumiants.

Early studies (Loosli and Harris, 1945; Lofgreen *et al.*, 1947) indicated that methionine improved the utilization of urea nitrogen in growing lambs. Later work has shown that the beneficial effect of the methionine supplementation was probably due to the sulfur present in its molecule, and since then it is been proved that the rumen microflora of sheep can utilize inorganic sources of sulfur in the synthesis of the sulfur containing amino acids (Block *et al.*, 1951; Gall *et al.*, 1951). Starks *et al.* (1953, 1954) demonstrated that elemental sulfur was used as effectively as sulfate or methionine sulfur when added to sulfur deficient diets for growing lambs. Some later work indicated that the sulfate sulfur was better utilized than the elemental sulfur and the reason was probably its better solubility (Hale and Garrigus, 1953; Albert *et al.*, 1959). Hume and Bird (1970) did not find any differerence in the microbial production when either cysteine or inorganic sulfate were added to sulfur deficient diets.

The sulfur requirement for growing lambs is reported to be about 0.08 to 0.10 percent of the diet dry matter (Reid, 1953; N. R. C., 1964). Many authors have indicated that the sulfur requirements should be expressed in relation to the nitrogen. In an early review of the sulfur requirements for ruminants Loosli (1952), indicated that the optimal ratio between nitrogen and sulfur should be close to that found in the tissue proteins of 15 parts of nitrogen to one of sulfur (15 : 1). Later work seems to indicate that a lower ratio will be as efficient, and Rozgoni (1960) recommended a ratio of 8.2 : 1. Moir *et al.* (1967) indicated that ratios from 9.5 : 1 to 12 : 1 are adequate for optimal nitrogen utilization. Allaway (1969) indicated that the optimum values for this nitrogen to sulfur ratio in ruminants ranged from 10 : 1 to 15 : 1.

It is generally accepted today that the inorganic sulfate has to be reduced to sulfite and sulfide before its incorporation into the sulfur containing amino acids (Lewis, 1954; Henderick, 1961; Halverson *et al.*, 1968). Methionine (Lewis,1955) and cysteine (Lewis, 1954; Anderson, 1956; Hume and Bird, 1970) have also been shown to be reduced to sulfide by the rumen microorganisms. Bosman (1966) reported a high concentration of sulfide in the rumen of cattle after the ingestion of a highly digestible protein. All these data tend to support the idea that the rumen microbes build all, or almost all, of their amino acids *de novo* (Walker and Nader, 1968).

There are conflicting results in the literature as to how many species of microorganisms are involved in the utilization of sulfide as a source of sulfur. Early *in vitro* work (Emery *et al.*,

1947a, 1957b) indicated that only a few species of microbes were able to incorporate the inorganic sulfate into the microbiad protein. HUNGATE (1966) indicated that: «it is highly probable that hydrogen sulfide can be used as a source of sulfur by most rumen microorganisms.»

WHANGER and MATRONE (1965, 1966 and 1967) and WHANGER (1968) reported the existence of significant changes, both quantitative and qualitative, in the microbial population in the rumen of sheep fed sulfur deficient diets when compared to animals receiving adequate sulfur diets. They also reported higher propionate and lower butyrate concentration in the rumen fluid of lambs fed the deficient diets than in those eating normal diets. There was also a high accumulation of lactate in the rumen fluid of the lambs receiving the deficient diets.

Most of the work reported has been done using purified diets in order to be able to provide very low levels of sulfur. Under normal feeding conditions sulfur deficiency is rather unlikely to occur. There are several reports in the literature indicating that hte sulfur supplementation of natural diets did not have any effect on nitrogen utilization (LOFGREEN *et al.*, 1953; DAVIS *et al.*, 1954; SLEN and WHITING, 1955) of diets that were not deficient in sulfur. DEIF *et al.* (1970) report that the addition of sulfur to a wheats traw diet containing about 0.14 percent sulfur and a N : S ratio of 9.5 : 1, increased nitrogen utilization. ALLAWAY (1969) indicated that some new fertilizing practices may eliminate some source of sulfur for the plants growing under those conditions and their sulfur content may be substantially reduced.

Summarizing we can say that there is no evidence in the literature indicating any changed mineral requirement for animals fed urea diets (BRIGGS, 1967). However, it is a well known fact that proteins are a good source of mineral elements and their removal from the diet also removes the mineral elements and these should be added back, especially when we use NPN compounds as a source of nitrogen instead of natural proteins (ARMSTRONG and TRINDER, 1966).

Urea for Lactating Animals.

There is good evidence in the literature that urea can successfully replace a part of the natural protein in the rations for dairy cattle. ARCHIBALD (1943), fed dairy cows rations containing up to three percent of the grain mixture as urea and found that the animals did not produce as well as those animals being fed the natural proteins, although the animals made considerable use of the urea in the ration. RUPEL *et al.* (1943), suppplementing dairy rations with urea at levels of one percent of the concentrate, report that the urea rations were equal to those containing linseed meal when measured as milk yield. Some later work (THOMSON *et al.*, 1952), indicated that urea was comparable to cottonseed meal when included in dairy cows' rations at levels up to three percent of the concentrate mixture, representing about 29 percent of the protein in the concentrate. LASSITER *et al.* (1958), supplemented rations with three levels of urea (30, 50 and 70 percent of the total nitrogen) and reported a slight but non significant decrease in milk production with increased levels of urea.

BALCH and CAMPLING (1961) in balance trials using a basal diet very low in protein and high in carbohydrate, which they supplemented with urea to make up to one percent of the dry matter intake, representing about 38 percent of the nitrogen, reported that the nitrogen from urea was almost completely utilized by the lactating cows.

HUBER and SANDY (1965) reported that with dairy cows fed three levels of concentrate (2.99, 6.58 and 10.52 kg. per day) with three levels of urea (0, 20 and 40 percent of the total nitrogen) with corn silage as the only forage, there was a trend toward decreased efficiency with increased levels of urea. At the high concentrate levels, urea significantly decreased the silage intake.

HOLTER *et al.* (1968) reported that dairy rations supplemented with urea up to 1.5 percent of the concentrate dry matter, were able to support an average milk yield of 24 kg. per day during 305 days. This is higher than any production reported previously. These results indicate that the performance of high producing dairy cows fed a palatable high quality mixture is not affected by supplementing it with urea up to 1.5 percent of the concentrate (HOLTER *et al.*, 1968).

Definitive evidence of the ability of urea to support milk production has been obtained in studies using purified diets in which almost all the nitrogen was given as NPN (VIRTANEN, 1966; FLATT *et al.*, 1969). These authors report milk yields of about 2.600 kg. in 305 days of

lactation. The milk produced by the cows on the purified diets did not differ from that of cows being fed rations containing natural proteins instead of the NPN compounds either in chemical composition or in flavor. The work of FLATT *et al.* (1969) tends to indicate that the inclusion of a small amount of protein in this type of diets is highly beneficial in terms of milk production. VIRTANEN (1967) estimated that lactating cows are able to produce up to 4.000 kg. of milk per lactation with NPN as the only source of nitrogen.

There is not much work done with lactating ewes fed NPN compounds as a source of nitrogen. Early work done by POPE *et al.* (1952, 1953), indicated that urea can be used to substitute part of the protein in the ration of lactating ewes. These authors used a basal diet with a protein level of ten percent and they added urea to increase this protein content to fifteen percent. Urea represented about two percent of the total dry matter of the diet. No significant differences were observed in the ewe's body weight changes during pregnancy and first six weeks of the lactation when either the cottonseed or the urea ration was fed.

PALIAN and MARKOTIC (1962), reported that urea was a suitable source of protein for lactating ewes, and they indicated that no differences were found in milk yield between the urea diet and a diet containing natural protein. KURLEC (1959) and SATTAROV (1965) indicated that urea could be successfully used in wintering rations for pregnant and lactating ewes fed poor quality forages. Urea and SBM supplemented rations fed to ewes resulted in no significant differences either in the ewes' body weight changes during gestation or in the percent of lambs born and weaned (JORDAN, 1952).

All the work reported has been done with lactating ewes suckling single lambs, and very little information can be found in the literature dealing with ewes suckling twin lambs. In general, there is very little information about the requirements of these animals and more information will be necessary in the future because these highly producing animals obviously cannot be successfully fed following the recommendations and requirements established for the ewes suckling single lambs.

EXPERIMENTAL OUTLINE

The present Thesis will be mainly concerned with the study of the value of urea as a source of nitrogen for both growing lambs and lactating ewes suckling twin lambs. The experimental work has been divided in two different experiments called hereafter Experiment 1 and 2. Experiment 1 will include the growing-finishing lambs, while Experiment 2 will cover the lactating ewes data.

When urea is substituted for soybean meal (SBM) in a ration at an isonitrogenous rate, this is usually done feeding a corn plus urea supplement. Because of the differences in mineral composition between the corn and the SBM, the urea diets may be deficient in some mineral elements, and this may become an important limiting factor when urea supplies most of the nitrogen in the ration. It was decided to perform an alalysis to know the content of SBM and corn samples in several minerals (K, S, Mn, Cu and Fe). The results of this analysis showed that SBM contained 2.24 % K, 0.353 % S, 40 pp. Mn, 31 ppm. Cu and 114 ppm. Fe, while the values for corn were, 0.35 % K, 0.081 % S, 0.24 ppm. Mn, 7 ppm. Cu and 58 ppm. Fe.

In Experiment 1, these minerals were added to urea diets to equate them to isonitrogenous SBM diets, and a growth and metabolism trials were carried out with growing lambs fed the diets at two protein levels (12 and 16 %). The two trials are described in the Experimental Procedures section.

Experiment 2 will be concerned with the study of the value of urea as a nitrogen supplement for lactating ewes when used as a source of nitrogen in rationes with different protein equivalents and different energy concentrations. The possibility of improving the urea value by «adapting» the ewes to the urea feeding during the last part of the gestation will also be studied in this Experiment. Finally, the possible effects of supplementing lactating ewes rations containing urea with the same minerals studied in Experiment 1, will also be studied here. This Experiment will be divided into three different trials which are described in the corresponding section of Experimental Procedures.

Experiments 1 and 2 will be described separately and at the end a general summary will summarize the results of both experiments. Since the same chemical determinations were often performed in the different trials, it is felt that grouping and describing all the analytical determinations for the two experiments in a common section will be helpful and will avoid unnecessary repetition.

CHEMICAL ANALYSES

The dry matter and ash of the feed, feces and refusal samples, were performed according to the Official methods of the A. O. A. C. (1955).

Total nitrogen in the feed, feces, refusals and urine samples was done following the Official A. O. A. C. (1955) method with the boric acid modification as proposed by SCALES and HARRISON (1920).

Blood Samples

Blood Hemoglobin.

Total hemoglobin was performed on the whole blood sample according to the method described by SANFORD and SHEARD (1929), except that the standardization was done according to the procedure of Wong (1928).

Plasma Proteins.

This analysis was performed by a biuret method as indicated by GORNALL *et al.* (1949) after the plasma was obtained by centrifugation of the blood samples at about 15.000 × G, for 15 minutes.

Protein Free Filtrate. (PFF)

The PFF was done using Na_2WO_2 and H_3SO_4 as proposed by FOLIN and WU (1919).

Blood Urea Nitrogen. (BUN)

The BUN analysis was done according to the colorimetric procedure described by COULOMBE and FAREAU (1963), after obtaining the PFF as described.

Blood Ammoia Nitrogen.

Blood ammonia nitrogen was determined in the PFF by a colorimetric method using the catalyzed indophenol reaction as described by CHANEY and MARBACH (1962).

Rumen Samples

Rumen Fluid Ammonia.

The ammonia concentration in the rumen fluid was performed by the method of CHANEY and MARBACH as described for the blood samples. The samples were previously strained through cheese cloth, and the protein was precipitated out with a ten percnet solution of TCA, followed by centrifugation at 15.000 × G for 15 minutes; this procedure was repeated three times.

Volatile Fatty Acids. (VFA)

The rumen VFA concentration was measured on a Perkin-Elemer 881 gas chromatograph using a 6' × 1/8" O. D. column packed with 20 percent Lac-1-R-296 on Chromsorb W 60/80 mesh, N. P. (Analabs). The injection block was maintained at 250 C and the detector system was kept at 185 C. A column temperature of 120 C was used with a helium gas carrier gas flow rate of about 120 ml./min. The strained rumen samples were prepared for the gas liquid chromatography analysis by the method of ERWIN (1961), except that the samples were centrifuged for 15 minutes at about 15.000 × G.

Milk Samples

The total nitrogen in the milk samples was done following the procedure indicated for the feed samples.

The dry matter of the milk samples was determined after the samples were freeze-dried for about 96 hours in a large freeze-dryer (Stokes, Model 2.004 L × B).

Milk fat was done according to the Babcock method (A. O. A. C., 1955), except that the milk was previously diluted with water in a one to one ratio due to the high fat content of the ewe's milk.

Lactose was performed following the method described by FOLIN and WU (1920).

STATISTICAL ANALYSES

The data were analysed by the analysis of variance (AOV) according to the procedures described by STEEL and TORRIE (1960). When the F values were significant at the five percent level of probability, the differences between the treatments were established by using either Duncan's multiple range test or the Dunnett's procedure (dsd) as described by Steel and Torrie.

Regression and correlation analyses, when performed, were done according to the procedures described by DRAPPER and SMITH (1966) and STEEL and TORRIE (1960).

EXPERIMENT 1.

Mineral Additions to Urea Diets for Growing Lambs.

Objectives.

The primary objectives of this experiment were:

a) to compare the value of urea versus soybean meal (SBM) for growing lambs fed two different protein levels,

b) to evaluate the effect of certain minerals (K, S, Fe, Cu, and Mn), lower in corn meal than in SBM, when added to urea diets to equate an isonitrogenous SBM control diet.

This experiment consisted of a feeding trial and of a metabolism trial called hereafter Trial 1 and 2, respectively.

Exprimental Procedure.
Trial 1.

Animals.

One hundred and forty four «native» feeder lambs with initial liveweights ranging from 21.8 to 35.4 kgs. were used in this trial. Seventy one of the lambs were males and seventy three were females.

Rations.

The different rations fed in this trial are showed in Table 1. All of the components in these rations were ground and thoroughly mixed and later fed as a complete ration. In order to get a better mixing of the different suppelemnts they were premixed with some corn meal and added to the mixer at the time the rest of the components were being mixed together. After the rations were sufficiently mixed, they were bagged, labelled and stored until used in the experiments. Representative samples of the different rations were taken for chemical analysis.

TABLE 1.

Experiment 1. Trial 1. Experimental Rations.

Ingredient	Treatments I	II	III	IV	V	VI
Hay (kg.)	50.0	50.0	50.0	50.0	50.0	50.0
Corn (kg.)	38.0	46.4	46.4	28.0	44.8	44.8
SBM (50 %) (kg.)	10.0	—	—	20.0	—	—
Urea (262) (kg.)	—	1.6	1.6	—	3.2	3.2
T. M. S.* (kg.)	1.0	1.0	1.0	1.0	1.0	1.0
Dicalcium phosphate (kg.)	1.0	1.0	1.0	1.0	1.0	1.0
$CuSO_4 . 5 H_2O$ (gm.)	—	—	.987	—	—	1.97
$MnSO_4 . H_2O$ (gm.)	—	—	.612	—	—	1.22
$FeSO_4$ (85 %) (gm.)	—	—	2.09	—	—	4.19
$KHCO_3$ (kg.)	—	—	.499	—	—	.996
Na_2SO_4 (kg.)	—	—	.124	—	—	.247
Crude Protein (%)	11.35	11.57	11.77	16.21	15.80	16.28
Estimated N:S	15.6:1	20:1	15.6:1	17:1	27:1	17:1

•Trace Mineral Salt, containing not more than 99.0 % NaCl and no less than (%) 96.0 NaCl, .20 Mn., .15 Fe, .10 Mg., .05 sulfate sulfur, .03 Cu .01 Co, .008 Zn and .007 I.

Experimental Design.

A 2 × 3 factorial arrangement of treatments in a randomized block with two protein levels, 12 and 16 percent, and three different supplements, SBM, urea and urea plus the mineral mixture, for a total of six treatments were used in this trial.

The 144 lambs were divided according to sex and initial liveweight into six experimental groups of 24 lambs each, and the six experimental treatments were randomly allocated to these groups. The trial lasted until the lambs reached 45 kgs. or after 105 days, whichever came first. Initial and final weights were taken after a 16 hour fast during which all feed and water were removed. The animals were also weighed weekly and these weights recorded. The lambs were «group-fed» and allowed to eat «ad libitum». The feed was offered twice daily at approximately

TABLE 2.

Experiment 1. Trial 1. Experimental Design.

	Protein level (%)					
	12			16		
Supplement	SBM	Urea	Urea & Minerals	SBM	Urea	Urea& Minerals
Group No.	I	II	III	IV	V	VI
No. of animals	24	24	24	24	24	24

8 AM and 4 PM. Water was available at all times and straw was used for bedding. Blood samples were taken from 14 lambs randomly chosen from each group. The blood was drawn by jugular puncture using a 14 gauge bleeding needle. The samples were taken at the beginning of the experiment and two, four, seven and ten weeks thereafter. The bleeding was usually performed at one PM and the samples were taken to the laboratory and analysed as soon as possible.

The different experimental treatments are shown in Table 2.

Chemical Analyses.

Total nitrogen was determined on the feed samples and Plasma Urea Nitrogen (PUN), plasma proteins and hemoglobin were performed on the blood samples according to the techniques previously described.

Trial 2.

Animals.

Twenty crossbred yearling wethers with liveweights ranging from 32.9 kgs. to 44.7 kgs., were used in this trial. All animals were fitted with a rumen canula.

Rations.

The five experimental rations fed in this experiment are shown in Table 3. The basal diet was similar to that of group II from the previous trial (Table 1). The four ohter diets consisted of supplementing this basal ration with different minerals as follows: a mixture of K, S, Cu, and Mn (Minerals); K alone (K); S alone (S) and a mixture of Cu plus Mn (Cu + Mn).

TABLE 3.

Experiment 1. Trial 2. Experimental Rations.

Ingredient	Treatments				
	Basal	Mineral	K	S	Cu + Mn
Hay (kg.)	50.0	50.0	50.0	50.0	50.0
Corn (kg.)	46.1	46.1	46.1	46.1	46.1
Urea (262) (kg.)	1.94	1.94	1.94	1.94	1.94
TMS* (kg.)	1.0	1.0	1.0	1.0	1.0
Dicalcium phosphate (kg.)	1.0	1.0	1.0	1.0	1.0
$CuCl_2 . 2 H_2O$ (gm.)	—	.81	—	—	.81
$MnCl_2 . 4H_2O$ (gm)	—	.86	—	—	.86
$KHCO_3$ (kg.)	—	.598	.598	—	—
Na_2SO_4 (kg.)	—	.152	—	.152	—

* Trace Mineral Salt.

The same hay and corn meal used in the previous trial were used for this one also and the rations were ground, mixed and fed to the animals in a similar way to that described for trial 1.

The lambs of the different groups were fed a prescribed amount of dry matter per unit metabolic body size (MBS) based on the maintenance requirements expressed as metabolizable energy (ME) per unit MBS per day as observed by PALADINES (1963), BURTON (1967) and BULL (1969). The ME value of the different components of the diets was estimated from the values published by the NRC (1964).

Every meal for the total duration of the trial was weighed and stored in small plastic bags which were properly labelled and kept in large garbage cans. Each daily ration was divided into two equal meals and one bag was used for each animal and meal.

Experimental Desing.

The 20 wethers used in this trial were divided according to their initial liveweight into five different groups of four lambs each. The five experimental rations were then randomly allocated to each of these groups. The animals were kept in metabolic crates allowing for separate collection of feces and urine. The daily meals were offered at 8 : 00 AM and at 4 : 30 PM. Water was offered in buckets twice daily inmmediately before the meals.

The animals were adapted to the metabolism cages for a period of ten days previous to the trial. During this adaptation period all lambs were fed a ration of ground hay and corn meal in a 50 : 50 ratio and fed as a complete ration. All lambs received about 600 gm. of this ration daily in two equal meals one in the morning and the other in the afternoon.

After this adaptation period the animals started receiving the experimental rations and the trial went as follows: a) a preliminary period of 10 days followed by b) a collection period of seven days which will be called «Period 1» hereafter; c) a rest period of seven days, during which the animals received the diets but no collection of excreta was done, and d) a second collection period of seven days, that hereafter will be called «Period 2.»

Sampling.

Feeds and Refusals.

At the time of preparing the daily meals for the different lambs a representative sample of each of the experimental diets was obtained. This sample was ground to pass through a 1 mm. screen in a Wiley mill. The material so ground was mixed and subsampled into identified glass bottles of about 100 gm. capacity. The same procedure was followed for the refusals if there were any present.

Feces and Urine.

The total amount of feces voided daily was weighed within the nearest gram, mixed and an aliquot representing the 25 percent of the daily total was put into a plastic container large enough to hold the samples for the seven days of the collection of each lamb. These plastic containers were kept in a deep freezer at-27 C until the end of the trial. When the trial was ended the containers with the frozen samples of feces were taken to a large freeze-dryer (Stokes, Model 2.004 L × 3) and dried for about 114 hours and then removed and the dry matter was determined by difference between the original and the final weights.

The dry feces were ground to pass a 1 mm. screen of a Wiley mill and this finely ground material was mixed and subsampled in the same way as described for the feed samples.

The urine voided daily was collected in plastic bottles to which 10 gm. of oxalic acid was added in order to acidify the urine and avoid nitrogen losses. The urine collected daily was weighed within the nearest gram and an aliquot representing 50 percent of the total amount was transferred to another plastic bottle and kept in a cooler at 2 C until the end the collection period, when each bottle was thoroughly mixed and subsampled into a labeled plastic bottle of approximately 100 ml. capacity. These samples were frozen and stored at -27 C until used for the chemical analysis. In the second collection period the size of the aliquot taken for the urine samples was reduced to 25 percent for easier handling.

Rumen Samples.

The rumen samples were obtained through the rumen cannula on the days 14 and 28 (days 7 of periods 1 and 2 respectively) of the trial. A plastic tube od about 12 mm. in diameter was used for obtaining the samples. The vacuum was provided by a small electrical vacuum pump. Immediately after removal of the sample it was passed through four layers of cheese cloth, to remove the gross feed particles present, and the samples were put into small plastic bottles containing 0.5 ml. of a 25 percent sulfuric acid togther with a small amount of mercuric chloride. The samples were frozen and kept this way until analysed in the laboratory.

Rumen samples were taken immediately before the morning feeding (time zero) and one, two, four and six hours later.

Blood Samples.

Blood samples were taken at the same time as the rumen samples. On the morning assigned for the bleeding, the lambs were fitted with a Jelco I. V. catheter placement unit 15 g × 6» introduced into the external jugular of the animals, where it remained until the last sample was taken and the catheter was removed. The samples were drawn with the help of a 10 ml. disposable syringe and transferred

into a small centrifuge tube containing some heparin to prevent clotting. The blood samples were kept in a cooler at 2 C and at the end of the day were taken to the laboratory and analysed as soon as possible.

Weghing.

The lambs were weighed at the beginning of the preliminary period and at the end of the trial. In both occasions the weights were taken immediately before the morning feeding.

Chemical Analysis.

The following analyses were done according to the methods already described in a previous section:

Feed, refusals and feces samples: Dry matter, ash and nitrogen
Urine: total nitrogen.
Rumen samples: ammonia nitrogen, Volatile Fatty Acids (VFS)
Blood samples: blood urea nitrogen (BUN).

RESULTS AND DISCUSSION

Trial 1.

Weight Gains, Feed Intake and Feed Efficiency.

The average daily gains (ADG), initial and final average weights, average feed intake and feed/gain of the animals in the different experimental groups are presented in Table 4.

TABLE 4.

Experiment 1. Trial 1. Average Initial and Final weights, Average daily gain, Feed efficiency and Estimated daily intake.

Variable	Treatments						
	I	II	III	IV	V	VI	$S_{\bar{x}}$**
No. of animals.	24	21	24	24	24	24	—
Ave. Init. wt. (kg)	28.5	28.9	28.8	28.8	28.9	28.9	.58
Ave. final wt. (kg)	42.2	39.6	41.7	43.4	39.6	42.0	.56
ADG* (gm)	141b	109c	130b	169a	105c	131b	.56
Feed Efficiency	9.6	11.9	11.3	9.0	12.2	10.7	—
Estimated intake kg./day	1.4	1.3	1.4	1.5	1.3	1.4	—

* Average Daily Gain
** n = 24
Values on the same line with different superscripts differ significantly (P < .05).

The lambs receiving the mineral supplemented diets had higher ADG ($P < .01$) than those receiving only the urea diets at both nitrogen levels. Increasing the protein content of the diets to 16 %, produced a significant increase in the ADG ($P < .01$) of the lambs fed the SBM ration, but no changes were observed in those lambs fed the urea diets. The lambs eating the mineral supplemented ration at the 12 percent crude protein equivalent, had similar gains to these in the SBM group and both groups had ADG significantly better ($P < .01$) than the urea group. The animals receiving the 16 percent CP diet supplemented with SBM, had higher ADG ($P < .01$) than any of the other experimental groups. The lambs fed the 16 percent urea diet supplemented with the minerals, had similar ADG to those receiving the 12 percent SBM and urea rations.

One lamb in group 2 died of unspecified causes after being on the experiment for seven weeks. The only symptoms observed were an extreme emaciation and weakness. The necropsy did not show any specific lesions. Two more animals had to be removed from the trial from group II, one after mine weeks and the other after 10 weeks. Both of them were losing weight and were extremely weak and thin at the time they were removed.

The feed intake per animal per day was estimated from the daily figures for the group intake and divided by the number of animals present at any moment in that specific group. The addition of the mineral supplements to the urea diets seems to have increased slightly the feed intake at both nitrogen levels. Increasing the amount of SBM in the diet slightly increased the feed intake.

The total feed per unit of gain (feed efficiency) was also estimated from the group intake figures and the total gain of the lambs on a group basis. The addition of the mineral supplements to the diets containing urea also produced a slight imrpovement in the feed needed per unit of gain. The removal of some animals from the group 2, makes the figures for this group of little value and diffcult to interpret.

Our results agree in general with the early work done by JOHNSON *et al.* (1942) and HAMILTON *et al.* (1948), who were unable to improve the nitrogen utilization by increasing the urea in diets with 12 percent crude protein equivalent up to 16 percent. When this increase in the protein content was done with linseed meal, the animals had an improved nitrogen retention. There are several reports in the literature tending to support the idea that lambs on urea diets do not perform as well as those on SBM (KAMMLADE *et al.*, 1940; NOEBE *et al.*, 1953, 1954, 1955; POPE *et al.*, 1953) or linseed meal (JOHNSON *et al.*, 1942; WILLMAN *et al.*, 1946; HAMILTON *et al.*, 1948).

The requirements for the minerals studied are shown in Table 5 and the calculated mineral composition of the diets is presented in Table 6. The differences in the mineral composition between the SBM and the urea diets is an indication that the removal of the protein supplement also takes away a good part of the mineral elements from the ration. It seems that our urea diets were satisfactory in manganese,

TABLE 5.

Mineral Requirement for Sheep.

Mineral	Requirement	Reference
Copper	4-10 mg/kg.	Allaway (1969)
	10 mg/kg.	Maynard & Loosli (1969)
Manganese*	16-25 mg/kg.	Rojas *et al.* (1965)
Iron	25-40 mg/kg.	Lawlor *et al.* (1965)
Potassium	.5 %	Telle *et al.* (1964)
Sulfur	.08-0.1 %	NRC (1964)

* Values for cattle.

TABLE 6.

Experiment 1. Trial 1. Estimated Mineral Composition of the different experimental rations.

	Rations*			
Mineral	I	II	IV	V
Copper (mg/kg.)	8.01	5.50	10.41	5.39
Manganese (mg./kg.)	41.62	39.64	43.22	39.25
Iron (mg./kg.)	83.44	76.91	89.04	75.98
Potassium (%)	1.09	.89	1.28	.89
Sulfur (%)	.12	.09	.15	.09

* Rations III and VI had the same mineral composition of rations I and IV respectively.

iron and potassium. The sulfur content was close to the minimum required and that together with an extremely wide nitrogen to sulfur ratio (20 : 1 for group II and 27 : 1 for group V) may have been a serious limiting factor in the nitrogen utilization in these two groups. The copper content of the urea diets was also on the low side of the minimum requiremnt for optimal growth, and according to the requirement listed, the urea diets may have been marginal in this element. Since these two elements have been shown to play an important role in the nitrogen metabolism (synthesis of sulfur containing amino acids and inhibition of the microbial urease) their inclusion in the mineral supplements of groups III and VI respectively may have played a role in the increased performance of these two groups. The addition of sulfur, besides raising its content in the diet to more acceptable levels, also reduced the wide nitrogen to sulfur ratio of these groups to 15.6 : 1 for group III and 17 : 1 for group VI, which still are considered wide, but it is felt that if the animals receive an adequate supply of sulfur they can tolerate quite wide nitrogen: sulfur ratios and still perform well. The copper present in the mineral mixture may have poduced a reduction in the concentration of the rumen fluid ammonia by decreasing the activity of the microbial urease (JONES *et al.*, 1964) which in turn would have resulted in a reduced rate of ammonia absorption and better utilization of the urea

nitrogen. The low levels of copper in the urea diets may have been deficient for the optimal growth of the microbial flora of the rumen and its addition may have helped establish a more adequate microflora needed for a better utilization of the urea nitrogen.

Besides the possible importance of the supplementation of the urea diets with these two potentially low mineral elements, the inclusion of the potassium bicarbonate may have also improved the buffer capacity of the rumen (BUNN and MATRONE, 1968) producing a slower absorption of the ammonia released and therefore a better nitrogen utilization.

There is also the possibility that the better performance of the mineral suplemented diets was not due to any such specific action of the minerals on the nitrogen metabolism, but to a more general action resulting in better growth.

The positive response obtained by the supplementation of the urea diets with the indicated mineral mixture seems to be a clear indication that one or more of the minerals in the mixture were limiting the optimal utilization of the urea diets for growth, but the fact that the group VI did not perform any better than group III or as good as IV seems to indicate that there are still some other factors, beside the minerals studied in this experiment, limiting the urea utilization.

Blood Hemoglobin, Plasma Proteins and Plasma Urea Nitrogen.

The average values for the plasma proteins, hemoglobin and plasma urea nitrogen (PUN) are presented in Table 7. Each value is the average of the values

TABLE 7.

Experiment 1. Trial 1. Average Plasma Proteins, Hemoglobin and Plasma Urea Nitrogen Concentrations and F values for the main factors and their interactions.

Treatments	Plasma Proteins. gm/100 ml.	Hemoglobin gm/100 ml.	PUN gm/100 ml.
I	6,62	11.59	11.48a
II	6.46	12.08	12.95b
III	6.46	11.81	13.80b
IV	6.44	11.75	16.40c
V	6.54	11.83	20.03d
VI	6.42	12.06	20.01d
s x	.02	.07	.18
F values			
Treatment	1.61 N. S.	1.25 N. S.	79.24***
Time	67.79***	3.05*	132.71***
Treatment × time	< 1 N. S.	< 1 N. S.	15.69***

Level of significance:
*** P < 005
** P < 01
* P < 05
N. S.—Non significant.

at different times of 14 lambs from each group. The F values of the different analyses of variance (AOV) for the principle factors and their interactions are presented in Table 7.

The plasma proteins and blood hemoglobin concentration were not affected by the different treatments and the values are within the normal range of values accepted for this specie (DUKES, 1955; ABOU AKKADA and EL-SHAZLY, 1965). The plasma protein concentration changed with time and this change was statistically significant ($P > .005$). The average values at the different times are shown in Table 8. After an initial drop in the plasma proteins concentration the values were back to the original values after four weeks and they continued increasing steadily and at our last sampling (week 10) they still seemed to be increasing. The initial drop corresponded with the phase of most active growth of the animals during the first two weeks of the experiment during which time a fairly large deposition of new tissue, protein, may be expected, and this probably would imply the use of all the nitrogen sources available to provide the amino acids needed.

This fact may explain the lower plasma protein concentration during the first two weeks of the trial. It remains a question of whether or nor the lower plasma proteins are indicative of a deficiency of protein during these moments of very fast growth and if it would be advisable to feed higher protein diets during the early phases of growth. Since in the later phases of the growth the protein deposition is greatly diminished and in its place there is an increased adipose tissue deposition, that may explain the rise in the plasma protein concentration.

Blood hemoglobin values also changed with time ($P < .05$) and the average concentrations for the different times are presented in Table 8.

TABLE 8.

Experiment 1. Trial 1. Average Plasma proteins and blood hemoglobin at the different sampling times.

Time (weeks)	Plasma Proteins gm/100 ml	Hemoglobin gm/100 ml.
0	6.25a	12.06b
2	5.99	11.62a
4	6.35a	12.05b
7	6.77	11.48a
10	7.09	11.98b

Values in the same column with different superscript differ statistically ($P < .05$).

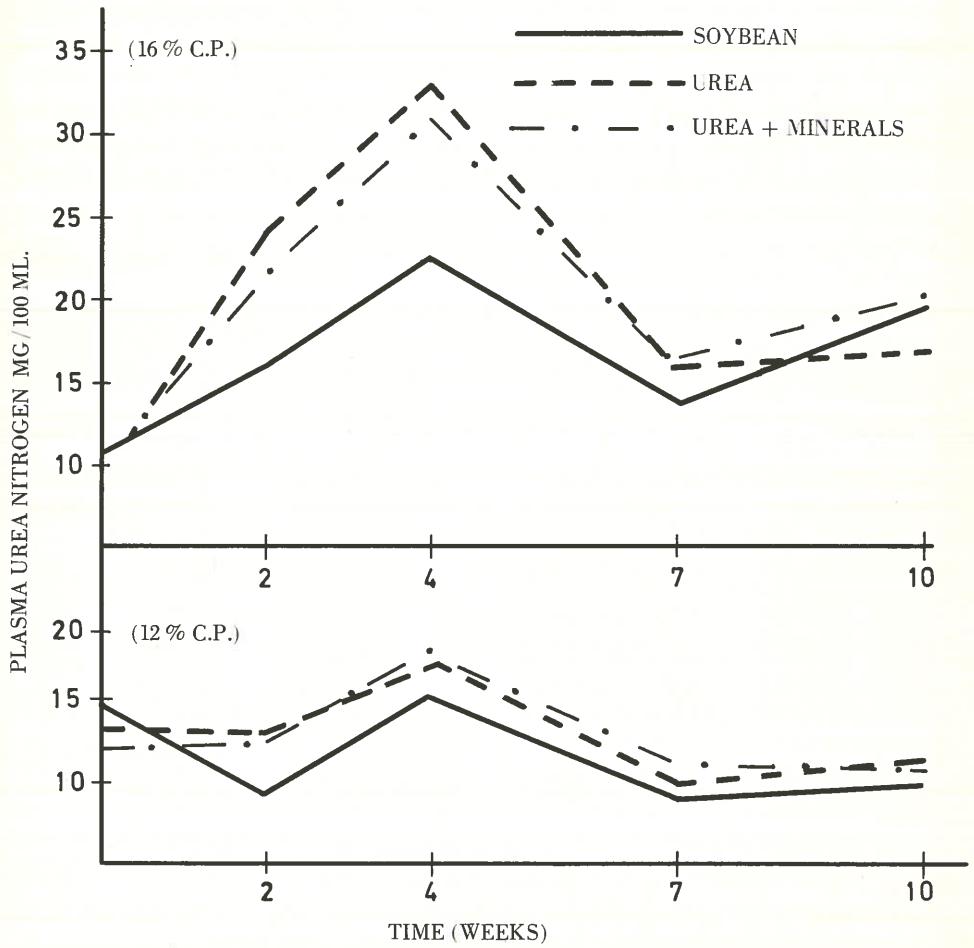
The average PUN concentration for the different treatments and the corresponding F values are shown in Table 7.

The lambs receiving the 16 percent crude protein diets, had higher PUN concentration ($P < .05$) than those eating the 12 percent diets. Within the two protein

levels the groups receiving SBM had lower ($P < .05$) PUN than those eating the urea diets. The mineral supplementation apparently did not have any effect on the PUN concentration at either of the two protein levels tested.

The time the animals were on the different diets had a significant effect on the PUN concentration ($P < .005$), and these changes of the PUN concentration with time are presented in Figure 1. The lambs eating the 16 percent crude protein rations supplemented with urea (groups V and VI), showed a faster increase with time in the PUN concentration than the lambs eating the SBM rations. The PUN values increased until the fourth week of the trial and thereafter they decreased and the values for both the urea and SBM diets came down to similar concentrations by

Figure 1. Experiment 1, Trial 1. Plasma Urea Nitrogen changes with time in the growing lambs.



the seventh week and from there on to the tenth week all three groups showed a small increase again. The picture for the groups receiving the 12 percent crude protein rations is very much like the one described, except that the rates of change are smaller and the differences between the SBM and the urea treatments, even though they still are statistically different, are much smaller.

These results agree with the early reports that indicated an increased PUN concentration when ruminants were fed NPN compounds (Dinning *et al.*, 1948; Repp *et al.*, 1955). There is now ample evidence in the literature that PUN concentration is primarily related to the rumen ammonia concentration (Lewis, 1957; Abou Akkada and El-Sayed Osman, 1967; Little *et al.*, 1968; Vercoe, 1969). Preston *et al.* (1965) reported that PUN values could be predicted from the protein content of the diet and they indicated that different proteins may have different equations but that the general shape of the different curves was expected to be similar. In a recent work using cattle as experimental animals Thornton (1970) reports that nitrogen intake strongly influences the rumen ammonia concentration and the PUN concentration. This author also indicated that there was a very high correlation between the PUN and the urinary urea excretion. Work done with different forages and protein sources (Tagari *et al.*, 1964; Abou Akkada and El-Sayed Osman, 1967) also indicated the existence of a close correlation between the nitrogen retention and the PUN concentration, and PUN is suggested as an index of the protein utilization of the ruminants.

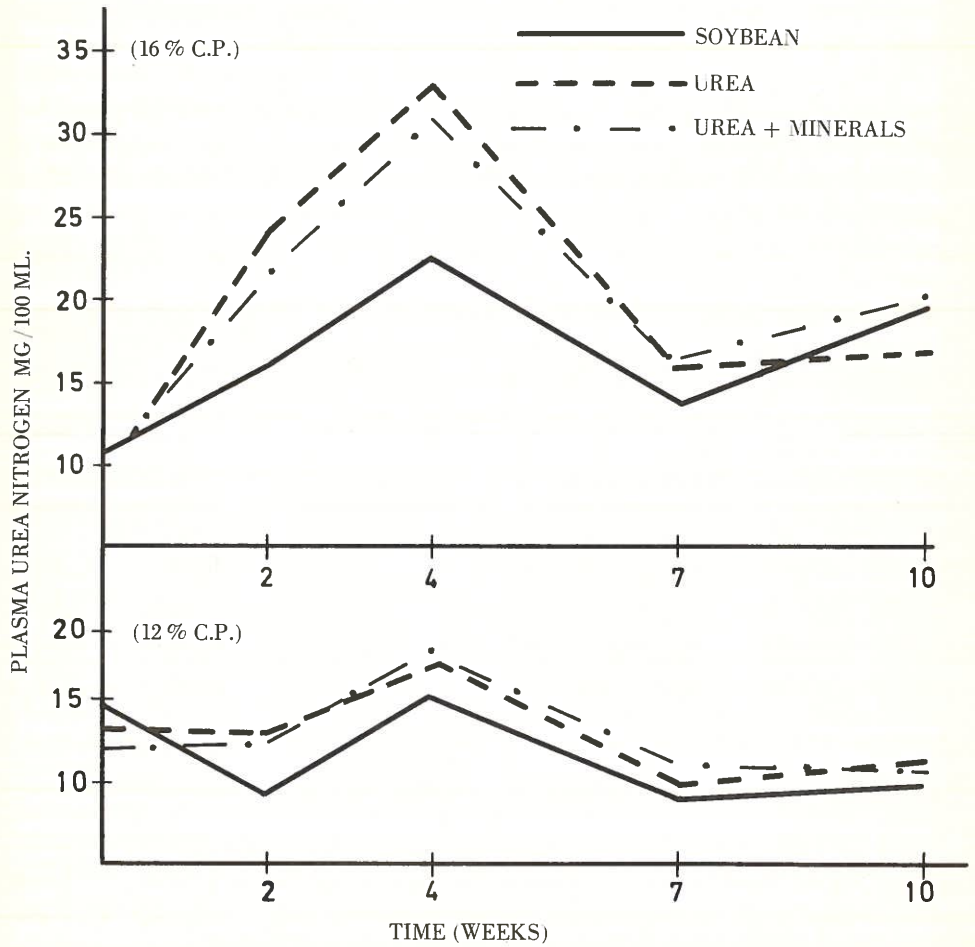
Our results agree in general with those of Abou Akkada and El-Sayed Osman (1967) and Littel *et al.* (1968) in that different nitrogen sources produce differences in the PUN concentration, most probably through differences in the rumen ammonia patterns. The high PUN values observed for the groups V and VI would indicate that a substantial amount of nitrogen may have been lost through the urine (Thornton, 1970). The later drop in the PUN values with time could be indicative that some type of adaptation had taken place, resulting probably in lower urinary losses and a better nitrogen utilization. The same will be true for the groups receiving the 12 percent crude protein, except that the changes taking place were smaller.

There are reports in the literature indicating that factors other than changes in the rumen ammonia concentration may be responsible for the changes in the PUN concentration both in cattle (Thornton, 1970) and in sheep (Sommers, 1961). Our results for the mineral supplemented groups would agree with these reports if we assume that the better performance of the animals receiving the mineral supplemented diets is due to an improvement of the nitrogen metabolism and more likely to an improved utilization of the ammonia nitrogen in the rumen, but these supposed changes did not get reflected in the PUN concentration. It is also possible that the improvement in the ADG of the lambs receiving the mineral supplemented diets was due to the presence of a better mineral balance and not by a direct action of

levels the groups receiving SBM had lower ($P < .05$) PUN than those eating the urea diets. The mineral supplementation apparently did not have any effect on the PUN concentration at either of the two protein levels tested.

The time the animals were on the different diets had a significant effect on the PUN concentration ($P < .005$), and these changes of the PUN concentration with time are presented in Figure 1. The lambs eating the 16 percent crude protein rations supplemented with urea (groups V and VI), showed a faster increase with time in the PUN concentration than the lambs eating the SBM rations. The PUN values increased until the fourth week of the trial and thereafter they decreased and the values for both the urea and SBM diets came down to similar concentrations by

Figure 1. Experiment 1, Trial 1. Plasma Urea Nitrogen changes with time in the growing lambs.



the seventh week and from there on to the tenth week all three groups showed a small increase again. The picture for the groups receiving the 12 percent crude protein rations is very much like the one described, except that the rates of change are smaller and the differences between the SBM and the urea treatments, even though they still are statistically different, are much smaller.

These results agree with the early reports that indicated an increased PUN concentration when ruminants were fed NPN compounds (Dinning *et al.*, 1948; Repp *et al.*, 1955). There is now ample evidence in the literature that PUN concentration is primarily related to the rumen ammonia concentration (Lewis, 1957; Abou Akkada and El-Sayed Osman, 1967; Little *et al.*, 1968; Vercoe, 1969). Preston *et al.* (1965) reported that PUN values could be predicted from the protein content of the diet and they indicated that different proteins may have different equations but that the general shape of the different curves was expected to be similar. In a recent work using cattle as experimental animals Thornton (1970) reports that nitrogen intake strongly influences the rumen ammonia concentration and the PUN concentration. This author also indicated that there was a very high correlation between the PUN and the urinary urea excretion. Work done with different forages and protein sources (Tagari *et al.*, 1964; Abou Akkada and El-Sayed Osman, 1967) also indicated the existence of a close correlation between the nitrogen retention and the PUN concentration, and PUN is suggested as an index of the protein utilization of the ruminants.

Our results agree in general with those of Abou Akkada and El-Sayed Osman (1967) and Littel *et al.* (1968) in that different nitrogen sources produce differences in the PUN concentration, most probably through differences in the rumen ammonia patterns. The high PUN values observed for the groups V and VI would indicate that a substantial amount of nitrogen may have been lost through the urine (Thornton, 1970). The later drop in the PUN values with time could be indicative that some type of adaptation had taken place, resulting probably in lower urinary losses and a better nitrogen utilization. The same will be true for the groups receiving the 12 percent crude protein, except that the changes taking place were smaller.

There are reports in the literature indicating that factors other than changes in the rumen ammonia concentration may be responsible for the changes in the PUN concentration both in cattle (Thornton, 1970) and in sheep (Sommers, 1961). Our results for the mineral supplemented groups would agree with these reports if we assume that the better performance of the animals receiving the mineral supplemented diets is due to an improvement of the nitrogen metabolism and more likely to an improved utilization of the ammonia nitrogen in the rumen, but these supposed changes did not get reflected in the PUN concentration. It is also possible that the improvement in the ADG of the lambs receiving the mineral supplemented diets was due to the presence of a better mineral balance and not by a direct action of

these minerals on the nitrogen metabolism. In any case the experimental design utilized in this trial did not allow us to determine which one of these possibilities may have been true.

Trial 2.

The chemical composition of the diets used in this second trial is shown in Table 9 and the averages for daily intake and initial and final weights of the animals is presented in Table 10.

TABLE 9.

Experiment 1. Trial 2. Chemical Composition of the Experimental Rations.

Rations	Dry Matter (%)	Ash (% DM)	Nitrogen (% DM)	N:S*
Basal	89.25	3.48	2.25	25.0:1.0
Minerals	89.68	5.61	2.29	10.0:1.0
Potassium	88.74	5.30	2.28	25.3:1.0
Sulfur	89.70	5.70	2.52	10.0:1.0
Copper + Manganese	89.73	3.50	2.44	27.1:1.0

* Estimated values.

TABLE 10

Experiment 1. Trial 2. Average Initial and Final Weights, Dry Matter Intake and Digestibility of the Organic Matter.

	Treatments					
	Basal	Minerals	K	S	Cu + Mn	s $\bar{x}$
No. Animals	4	4	4	4	4	...
Ave. Init. Wt. (kg.)	37.6	37.7	37.4	37.7	38.3	0.64
Ave. Final Wt. (kg.)	37.3	38.2	38.2	38.9	38.2	0,70
DM intake (g./day)	600	604	594	604	607	7.54
OM digestibility (%)	70.82	70.04	69.29	72.53	71.40	0.32

Organic Matter Digestibility

The addition of the different minerals to the urea diet did not produce any significant changes in the digestibility of the organic matter and meither did the organic matter digestibility change with time. The average values of the organic matter digestibility are presented in Table 10.

Rumen Ammonia.

A summary of the rumen ammonia values is presented in Table 11. The Addition of copper and manganese to the urea diet, produced a lower rumen

TABLE 11.

Experiment 1. Trial 2. Average Rumen Ammonia concentration (mM/l)

	Treatments				
	Basal	Minerals	K	S	Cu + Mn
Period 1:					
feeding time	5.70	4.95	4.55	4.72	3.50
1 hour after feeding	21.05	17.92	15.82	17.72	18.72
2 hr. after feeding	18.80	18.40	17.10	17.12	15.82
4 hr. after feeding	9.12	8.95	8.74	6.73	5.63
6 hr. after feeding	6.05	4.33	4.30	4.30	2.93
average	12.14	10.91	10.13	10.12	9.32a
Period 2.					
feeding time	3.43	2.90	2.40	3.05	3.43
1 hr after feeding	13.15	13.12	10.45	13.55	14.65
2 hr after feeding	10.10	12.90	10.65	10.60	11.75
4 hr after feeding	7.20	6.80	5.08	5.98	5.52
6 hr. after feeding	4.40	4.55	3.25	2.83	2.45
average	7.66	8.06	6.37	7.20	7.56

Menas in the same line with different superscripts differ statistically ($P < .05$).

ammonia concentration ($P < .05$) after 14 days on the experimental diets. The other treatments also produced a slight reduction in the rumen ammonia concentration after 14 days but these reductions were not statistically different from the values obtained for the urea diet.

The rumen ammonia concentration reached its peak sometime between one and two hours after feeding and then decreased to values similar to those at feeding time after six hours. After 28 days on the experimental diets, the rumen ammonia concentration was lower ($P < .001$) than at 14 days, and this was true for all treatments. This reduction of the ammonia concentration with time was of the order of 30 percent as average across the treatments. There were no significant differences among the different treatments after receiving the experimental diets for 28 days.

The evolution of the rumen ammonia concentration with time was similar to that observed after 14 days, except that the peak values seem to have been reached one hour after feeding.

No similar values have been found in the literature with which to compare our results, but in general the evolution of the rumen ammonia concentration with time is similar to the values reported for urea diets both in steers (Chalupa *et al.*, 1964) amd sheep (Caffrey *et al.*, 1967), and indicate a fairly fast hydrolysis of the urea in the rumen after ingestion of the feed. Caffrey *et al.* (1967) have reported that maximum ability of the ruminal microorganisms to assimilate ammonia was reached before the lams had been fed 19 days on a urea diet, and that the microbial adjustment to the urea diets was accomplished before 13 days on the diet. If we assume that high rumen ammonia concentration is indicative of a poor ammonia

utilization, then we have to conclude that the lambs in our experiment were not completely adapted to the experimental diets after 14 days, and that between 14 and 28 days some further adaptation took place. This will be specially true for the animals in the basal group which showed the larger reduction in the rumen ammonia concentration with time. The addition of the different minerals seems to have helped in developing a better microbial population, and this is reflected in the lower rumen ammonia concentrations after 14 days on the diets.

The groups to which potassium was added (Minerals and K) maintained high ammonia values up to two hours after feeding while most of the other groups at two hours already were showing a decrease in the ammonia concentration.

The lower rumen ammonia concentration found when copper was added to the basal diet may have been due to its known antiurease activity. Even though the reduction in rumen ammonia concentration after 14 days when the others minerals were added to the urea diets was not statistically significant, it may be indicative of a response of the microbial population to somewhat better conditions for growth when these minerals were present in the rumen. The establishment of the mineral requirement for a maximum utilization of the ammonia nitrogen in the rumen remains to be done and these results are only indicative that under similar conditions the role of these minerals may be one important factor to take into consideration.

Rumen Volatile Fatty Acids (VFA)

A summary of the treatment means for the rumen VFA concentration is presented in Table 12.

The addition of the different minerals did not produce any statistically significant difference in the rumen VFA concentration after 14 or 28 days, however, the rumen VFA concentration of all treatments was higher ($P < .001$) after 28 days than after 14 days. The increase in the VFA concentration was of the order of 30 percent in the average for the different treatments.

The time after feeding had a significant effect on the rumen VFA concentration and since there were no differences among the treatments the different values were averaged across treatments and presented in Table 12. All three VFA measured seem to have reached the peak concentration two hours after feeding and thereafter they decreased to reach values similar to those obtained at feeding time after six hours.

When the VFA are expressed as a percentage of the total there were no significant differences among the different treatments at any of the two sampling periods. However, there were some differences between the samples taken at 14 days and those obtained after 28 days. The percentage of acetate increased slightly ($P < .05$) after 28 days, while the propionate concentration showed a small decrease after 28 days ($P < .001$). The butyrate did not suffer any variation with time. These

TABLE 12.

Experiment 1. Trial 2. Average Rumen VFA's concentration for the different treatments and times after feeding.

	Treatments				
	Basal	Minerals	K	S	Cu + Mn
Period 1.					
total VFA mM/L	77.1	81.0	69.8	75.5	64.9
individual VFA (mole %)					
acetic	65.9	66.9	65.2	68.6	65.5
propionic	20.7	21.7	22.2	19.9	22.9
n-butyric	13.3	11.7	12.7	10.9	11.6
Period 2.					
total VFA mM/L	108.1	97.5	105.0	104.4	97.8
individual VFA (mole %)					
acetic	66.2	67.3	68.7	68.1	68.4
propionic	20.0	18.3	19.0	19.7	20.9
n-butyric	13.7	14.4	12.3	12.2	10.7

	Time after feeding (hours)					
VFA (mM/L)	0	1	2	4	6	$s\bar{x}$
Period 1.						
acetic.	35.85	50.15	56.04	55.07	43.81	6.60
propionic	9.68	17.26	19.42	18.36	14.15	1.58
n-butyric	7.03	8.79	9.94	9.74	7.54	1.81
Period 2.						
acetic	51.47	73.38	85.78	75.07	60.76	—
propionic	13.16	22.17	24.93	22.68	17.29	—
n-butyric	10.62	12.71	14.64	14.53	11.39	—

changes in the proportion of the acetate and proprionate may tend to indicate a better utilization of the forage in the diet (cellulose).

These results will tend to agree with those of HUME and BIRD (1970) who did not find differences in the molar proportions of the individual VFA concentration when different sources of sulfur were added to low sulfur diets in which urea was the most important source of nitrogen.

BUNN and MATRONE (1968) observed changes in the rumen butyrate and acetate percentages in lambs fed purified diets with urea as the only source of nitrogen and supplemented with cations (sodium and potassium). These differences were found only when the animals were fed ad libitum. In our study the variation between aniamls within treatmnets was large enough to obscure any possible differences in the VFA concentration among the different treatments.

Blood Urea Nitrogen. (BUN)

The average BUN values for the two periods and the average for the trial are shown in Table 13. The different treatments did not produce significant changes in the BUN concentration. After 28 days the BUN values were higher ($P < .001$) than after 14 days. The BUN concentration increased with time after feeding, and six hours after feeding the values still were rising.

Because of operational difficulties with some of the catheters used to obtain the blood samples there were several missing observations that obviously make it difficult to interpret the results concerning the BUN.

The observed increase with time of the BUN concentration does not seem to go along with the rest of the results in the trial, and there is the possibility that factors other than rumen ammonia concentration may have influencied the BUN (SOMMERS, 1961). The evolution of the BUN values with time after feeding is in agreement with other reports in the literature that indicate that BUN reaches its peak some 6 to 8 hours after the rumen ammonia has done so (LEWIS, 1957; TAGARI *et al.*, 1964).

TABLE 13.

Experiment 1. Trial 2. Average BUN concentration values after 15 or 30 days on the rations.

Treatment	BUN (mg/100 ml)	
	Period 1	Period 2
Basal	47.0	44.4
Minerals	42.5	50.5
K	39.3	40.7
S	45.3	50.6
Cu + Mn	41.2	48.1

Nitrogen Balance.

The summary of the nitrogen balance data is presented in Table 14. The values for Period 1, correspond to the collection period between the days 10 and 17 and those for Period 2, to the collection performed between the 24th and 31st days.

After 17 days on the experimental diets there were no statistically significant differences in the nitrogen retained among the different treatments, however, the groups receiving the sulfur and copper plus manganese supplements had a slightly higher nitrogen retention than the basal urea group, but these differences were not statistically significant. We already have seen that these groups also showed a lower rumen ammonia concentration during the first sampling period (Table 11). After the lambs had been fed for 31 days on the experimental diets, the animals in the group supplemented with copper plus manganese, had higher nitrogen retention ($P < .01$)

TABLE 14.

Experiment 1. Trial 2. Nitrogen Balance Data.

	Treatments				
	Basal	Minerals	K	S	Cu + Mn
Period 1.					
Feed N (gm/day)	13.51	13.55	13.54	15.21	14.80
Fecal N (gm/day)	3.32	2.95	3.30	3.34	3.72
N absorbed % intake	75.42	78.22	75.64	78.02	74.90
Urinary N:					
gm/day	8.04	8.71	8.14	8.94	7.86
% intake	59.5	64.3	60.1	58.8	53.1
N retained					
gm/day	2.15	1.89	2.10	2.93	3.22
% intake	15.91	13.95	15.51	19.26	21.76
Period 2.					
Feed N (gm/day)	13.51	13.83	13.54	15.21	14.80
Fecal N (gm/day)	3.23	3.15	3.44	3.27	3.48
N absorbed % intake	76.09	77.22	74.59	78.38	76.48
Urinary N:					
gm/day	8.07	9.13	7.48	8.79	7.56
% intake	59.73	66.01	55.24	57.79	51.08
N retained					
gm/day	2.21	1.55	2.62	3.15	3.76
% intake	16.36	11.21	19.35	20.71	25.41

than those lambs on the basal diet. The potassium and sulfur groups also had a higher nitrogen retention than the basal group, but this increase did not reach statistical significance.

The performance of the group supplemented with all the minerals is difficult to understand and does not seem to go along with the results presented for the other treatments without any apparent reason to explain it.

The time the animals were fed the experimental diets did not have any statistical effect on the nitrogen retention of the different treatments. However, two of the treatments showed some substantial increment in the nitrogen retained with time (S and Cu + Mn groups) while the group with the minerals had a decrease in the nitrogen retained after 31 days on the experimental diet. The increase in the nitrogen retantion with time of the two mentioned groups, would agree with the early report of SMITH *et al.* (1960) indicating increases of 0.20 percent units per day up to 50 days. The major factor in the increased nitrogen retention seems to have been a reduction in the urinary nitrogen losses in these two groups. The lack of changes of the urinary nitrogen loss with time of the basal and S groups may have been the reason for the absence of any change in the nitrogen retained of these two groups with time.

CONCLUSIONS:

As a result of this study it can be concluded that:

1. The addition of minerals (Cu, K, S, Fe, Mn) to urea supplemented diets significantly increased the gains of growing finishing lambs.

2. The addition of Cu + Mn to a urea supplemented diet reduced the rumen ammonia concentration in lambs fed at maintenance level after 15 days on the diet.

3. The addition of Cu + Mn to a urea supplemented diet increased the nitrogen retention in lambs after 30 days on the ration.

4. Rations supplemented with SBM produced higher gains in lambs than isonitrogenous rations containing urea instead of the SBM, when the protein equivalent of the rations was of about 16 percent. Urea represented about the 52 percent of the total nitrogen in the ration.

EXPERIMENT 2.

Urea Supplementation for Lactating Ewes Suckling Twin Lambs.

Objetives.

The objectives of this study were the following:

1) To compare the value of urea and SBM at different protein levels in the rations fed to lactating ewes suckling twin lambs.

2) To evaluate the effect of «adaptation» to the urea by feeding a corn-urea supplement during the last 4 weeks of the gestation.

3) To evaluate the effect of the addition of minerals (potassium, copper, manganese, iron and sulfur) to urea supplemented rations in order to equal the mineral content of an isonitrogenous SBM ration.

4) To study the changes in some blood metabolites (hemoglobin, plasma proteins, blood ammonia nitrogen and blood urea nitrogen) in lactating ewes suckling twin lambs during the first six weeks of the lactation period.

In order to fulfill these objectives, three trials were carried out in as many consecutive years which hereafter will be identified as Trial 1, Trial 2 and Trial 3 respectively.

Experimental Procedures

Trial 1.

Animals.

Twenty six western ewes with initial weights ranging from 51.7 to 76.7 kg. were used in this trial. All ewes were suckling twin lambs sired by Hampshire rams.

Rations.

Four different rations were used in this trial, and they are described in Table 15. The hay used in the rations of this experiment was a poor quality grass hay (late first cutting, July 15-30), mostly composed of timothy (*Phleum pratense)* and orchardgrass (*Dactylis glomerata).*

The protein content of the hay used was 6.5 percent in trial 1, 7.7 percent for trial 2 and 9.0 percent in trial 3.

The rations were ground, mixed and sampled as described for Experiment 1(growing lambs). The rations were fed as a complete ration, and the hay to grain ratio was 40 : 60.

TABLE 15.

Experiment 2. Trial 1. Experimental rations

Ingredient	Rations			
	Basal	SBM	Low Urea	High Urea
Hay (Kg.)	40.0	40.0	40.0	40.0
Corn meal (Kg.)	58.0	48.0	56.4	54.8
SBM (Kg.)	—	10.0	—	—
Urea (Kg.)	—	—	1.6	3.2
TMS* (Kg.)	1.0	1.0	1.0	1.0
Decalcium phosphate (Kg.)	1.0	1.0	1.0	1.0
Crude Protein (%)	7.6	12.0	12.1	16.8

* Trace Mineral salt.

Experimental design.

A completely random design with five different treatments was used in this trial. The ewes in the first group were fed a basal diet made of hay and corn meal in the ratio of 40 : 60. This ration was intended to have a protein content of about eight percent. The animals in the second group received the same basal ration supplemented with SBM to have a protein content of about twelve percent. Ewes in the third group were fed the basal ration in which the SBM supplement was replaced by urea at a rate isonitrogenous with the SBM ration. The ewes in the fourth group were fed the same diet than those in the third group, but the ewes were «adapted» to the urea feeding during the last part of the gestation period. The fifth group was fed the basal ration supplemented with urea to have a protein equivalent of about sixteen percent. This experimental design is summarized in Table 16.

All the ewes used in this trial were shorn shortly before lambing, and during the last four to six weeks of the gestation period they were group fed long hay «ad libitum» and about 90 gm. of either SBM or an isonitrogenous corn-urea mixture per ewe per day. The ewes with twin lambs coming from the group fed the corn-urea

TABLE 16.

Experiment 2. Trial 1. Experimental desing.

	Experimental Rations*				
	Basal	SBM	Low Urea	Low Urea	High Urea
Protein equivalent (%)	8	12	12	12	16
Treatment	11	12	13	14**	15
No. of ewes***	5	5	4	8	4

* The rations are described in Table 15.
** «Adapted» ewes.
*** The different treatments had unequal numbers of ewes due to difficulties in obtaining enough ewes with twin lambs.

supplement, were later assignerd to the experimental treatment No. 13 (third group) that will be denoted «adapted» group.

The ewes were assigned to the different experimental groups the day after lambing, and the only consideration in grouping them was their weight and size at lambing time.

Within 24 hours after lambing, the lambs and the ewe were weighed and this weight recorded as initial weight. The ewes and the lambs were weighed weekly during the duration of the trial. All weights were taken at about the same time after the morning feeding. The ewes were kept in individual pens, 140 cm. × 130 cm., where they remained for the six weeks duration of the trial. The feed was offered twice daily at about 8 : 30 AM and again at 4 : 30 PM. Water was available at all times and wood shavings were used for bedding.

The ewes were fed to body weight maintemance throughout the trial, and the intake was adjusted individually each week according to the body weight changes of the previous week. The lambs were castrated and docked during the first two weeks of the trial.

Blood samples were taken from the ewes at lambing time and every other week thereafter until the sixth week. The blood samples were obtained by puncture of the jugular vein. All samples were taken at about the same time after the morning feeding (about 2 : 00 PM). The samples were taken into 50 ml. glass centrifuge tubes containing heparin to avoid clotting, and the chemical analysis were performed as soon as possible.

Milk production was estimated in all ewes according to the procedure reported by GARDNER and HOGUE (1964), consisting of hand milking at the beginning and end of a 2 1/2 hour interval, during which time the ewes were separated from their lambs. Milk let-down was artifically stimulated prior to each milking by intrajugular injection of five U. S. P. units of Oxytocin in five ml. of physiological saline. Milk samples were obtained during weeks three and five of the lactation. The samples were filtered through a glass fiber filter to remove all foreign material

and then were transferred to 100 ml. plastic bottles where they were stored frozen until analyzed in the laboratory.

Chemical Analysis.

Feed Samples.

Total nitrogen was determined in the feed samples as was previously described in Experiment 1.

Blood Samples.

Plasma proteins, blood hemoglobin, blood ammonia nitrogen and BUN were performed in the blood samples following the procedures already described in Experiment 1.

Milk Samples.

Dry matter, total nitrogen, percent fat and lactose were performed in the samples as described in the section Chemical Analyses.

Trail 2.

Animals.

Forty Western ewes with initial weights ranging from 44.4 to 70.8 kg., were used in this trial. All ewes were suckling twin lambs sired by Hampshire rams.

Rations.

The rations fed in this trial are described in Table 17. The rations were mixed, ground, sampled and fed as described for Trial 1. The rations of this trial had a hay to grain ratio of 50 : 50.

Experimental Design.

A completely randon design with five different treatments was used in this trial. The ewes in the first group were fed a corn and hay diet supplemented with SBM to have a protein content of about 18 percent (SBM ration from Table 17). The animals in the second treatment, were fed an isonitrogenous ration in which urea was substituted for SBM (Urea diet from Table 17). The ewes in the third group received the same rations as those in the second group but they had been «adapted» to the urea feeding during the last four weeks of the gestation period in a similar way to that described for Trial 1. The fourth and fifth groups were fed the same urea supplemented ration that was used for the previous groups but supplemented with

TABLE 17.

Experiment 2. Trial 2. Experimental Rations.

Ingredient	Rations		
	SBM	Urea	Minerals
Hay (kg.)	50.00	50.00	50.00
Corn (kg.)	24.00	44.10	44.10
SBM (kg.)	24.00	—	—
Urea (kg.)	—	3.89	3.89
$CuSO_4 . 5 H_2O$ (gm.)	—	—	1.97
$MnSO_4 . H_2O$ (gm.)	—	—	1.22
$FeSO_4$ (85 %) (gm.)	—	—	4.19
$KHCO_3$ (kg.)	—	—	996.44
Na_2SO_4 (kg.)	—	—	247.24
Trace Mineral Salt (kg.)	1.0	1.0	1.0
Dicalcium Phosphate (kg.)	1.0	1.0	1.0
Crude Protein (%)	18.2	19.1	18.8

minerals (K, Fe, Cu, Mn, S). This ration was isonitrogenous and had the same mineral content as that of the SBM ration used in this trial. The only difference between the fifth and the fourth groups was that the ewes in the former one had been «adapted» to the urea feeding. The experimental design for this trial is summarized in Table 18.

The grouping, weighing, sample collection and general animal management were performed in a similar way to that described for the Trial No. 1 with the exception that no milk samples were collected in this trial.

TABLE 18

Experiment 2. Trial 2. Experimental desing.

	Experimental Rations*				
	SBM	Urea	Urea	Minerals	Minerals
Protein equivalent (%)	18	18	18	18	18
Treatment No.	21	22	23**	24	25**
No. of ewes	8	8	7	10	7

* The rations are described in Table 17.
** «Adapted» ewes.

Chemical Analysis.

The same chemical determinations listed for Trial No. 1 were performed in the samples collected in this trial with the exception of the milk samples.

Trail 3.

Animals.

Thirty six Western ewes with initial weights ranging from 51.3 to 70.8 kgs. were used in this trial. All ewes were suckling twin lambs sired by Hampshire rams.

Rations.

Three different rations were fed in this Trial, and they are shown in Table 19. All rations had a hay: grain ratio of 40 : 60. The mixing, grinding, sampling and feeding of the experimental rations was similar to that described for Trial 1.

TABLE 19.

Experiment 2. Trial 3. Experimental Rations.

Ingredients	Rations		
	SBM	Urea	Minerals
Hay (gk.)	40.00	40.00	40.00
Corn (kg.)	40.00	55.08	55.08
SBM (kg.)	18.00	—	—
Urea (kg.)	—	2.92	2.92
$CuSO_4 . 5 H_2O$ (gm.)	—	—	1.77
$MnSO_4 . H_2O$ (gm.)	—	—	1.10
$FeSO_4$ (85 %) (gm.)	—	—	3.76
$KHCO_3$ (gm.)	—	—	896.78
Na_2SO_4 (gm.)	—	—	221.86
TMS* (kg.)	1.0	1.0	1.0
Dicalcium Phosphate (kg.)	1.0	1.0	1.0
Crude Protein (%)	17.71	17.91	17.95

* Trace Mineral Salt.

Experimental Design.

A completely random design with four experimental treatments was used in this Trial. The ewes in the first group were fed the SBM ration from Table 19. The ewes in the second group were fed an isonitrogenous ration in which urea was used instead of SBM, while the ewes in the third treatment received the same experimental ration but had been «adapted» on the same way described for the two previous trials. The ewes in the fourth group received a similar ration to that used in the previous groups but that had been supplemented with minerals (K, Fe, Cu, Mn, S) in order to equate the mineral content of the SBM fed in this Trial. A summary of this experimental design is presented in Table 20.

The general management of the animals and sampling procedures followed in this trial were similar to those described for Trial 1. No milk samples were obtained in this Trial.

TABLE 20.

Experiment 2. Trial 3. Experimental desing.

	Experimental Rations*			
	SBM	Urea	Urea	Minerals
Protein equivalent (%)	18	18	18	18
Treatment	31	32	33**	34
No. of ewes	6	10	10	10

* The rations are described in Table 19.
** «Adapted» ewes.

Chemical Analyses.

The feed samples were analysed as described for Trial 1.

Only blood urea nitrogen concentration was performed in the blood samples and the analysis was done as described elsewhere.

RESULTS AND DISCUSSION

Trial 1.

Milk Yield.

The average daily milk yield for the ewes in the different treatments is shown in Table 21. The ewes fed the basal ration yielded less milk ($P < .05$) in the third week of the lactation than those in the other treatments except the ewes fed the low urea diet. The ewes receiving the SBM supplemented ration had the highest milk yield, but it was not statistically different from the groups fed the high urea ration and the ewes «adapted» to the urea feeding. The variation between animals within treatments was large as reflected by the large standard errors. In the fifth week of lactation the situation was very much alike except that the total milk production was reduced. The ewes fed the high urea ration had smaller reduction in the milk yield (about eleven percent) after five weeks than the ewes in the other groups and especially those being fed the basal ration in which milk production dropped about 38 porcent. The other groups had intermediate reductions in the milk yield after five weeks.

TABLE 21.

Experiment 2. Trial 1. Average Milk yield of the ewes in the different treatments in the third and fifth weeks of the lactation.

	Average Milk Yield (gm/day) Weeks	
Treatment	3	5
Basal	1243.2	775.7
SBM	2593.9	1758.7
Urea	1706.9	1339.2
Urea «adapted»	2312.6	1665.0
High Urea	2174.4	1939.2
sx	235.8	247.6

Menas in the same column with different superscripts differ statistically ($P < .05$)

The milk yields obtained in this trial were lower than those reported by GARDNER and HOGUE (1964, 1966) for lactating ewes suckling twin lambs. Our ewes had larger reductions in the milk yield after five weeks than those reported by the mentioned authors.

Milk Composition.

No statistically significant differences were observed among the different treatments for any of the milk components studied. Because of this lack od differences, all values were averaged across treatments and the mean values are presented in Table 22. The F table for the different milk factors is shown in Table 23.

TABLE 22.

Experiment 2. Trial 1. Milk composition (1)

Dry Matter %	19.44 ± 2.86
Fat %	8.37 ± 2.67
Protein (N × 6.38) %	4.84 ± .57
Lactose %	5.03 ± .56

(1) Each value is the mean of 54 observations.

TABLE 23.

Experiment 2. Trial 1. F values for the milk studies

	Week	
Factor	3	5
Weight	7.24+++	4.62++
Dry Matter	<1 NS	<1 NS
Fat	<1 NS	<1 NS
Protein	<1 NS	<1 NS
Lactose	1.59 NS	1.60 NS

Level of significance.
+++ $P < 0.005$
++ $P < .01$
\+ $P < .05$
NS- non significant at $P < .05$.

The fat content of the milk was very variable with fairly large differences between animals that is reflected in the large standard error. In general the animals with lower milk yields had at the same time a higher fat content (%). This large variation in the fat content may help account for the variability observed in the dry matter content of the different milk samples. The average milk composition of our ewes agrees in general with the values reported by GARDNER and HOGUE (1964, 1966) for ewes suckling twins lambs.

Lamb Gains.

The mean values for the ADG and the total lamb production per ewe are shown in Table 24. As it could be expected, the growth of the lambs was closely correlated with the milk production of the ewes. The lambs in the SBM group had a higher ADG ($P < .05$) than those in the basal treatment. «Adaptation» of the ewes to the urea feeding increased the ADG ($P < .05$) of the urea treatment to values similar to those obtained for the SBM treatment. The low urea treatment did not improve the lambs ADG when compared to the basal group. The high urea treatment produced higher ADG ($P < .05$) than both the basal and low urea groups and similar to those reported for the SBM and urea «adapted» treatments.

TABLE 24.

Experiment 2. Trial 1. Average lamb gains, Average ewe weight changes, Average ewe feed intake and Average feed efficiency.

	Treatments				
	Basal	SBM	Urea	«Adapted»	High Urea
No. of ewes	5	5	4	8	4
Ave. lamb gain (kg)[1]	11.7	19.8	13.6	17.4	19.1
ADG (gm)[2]	139	204	161	207	228
Ave. ewe wt. change (gk)	-5.7	-1.4	-.9	-2.2	-5.0
Ave. daily ewe feed (kg.)	2.3	2.7	2.6	2.8	2.6
Total feed/lamb gain[3]	13.3	7.4	8.4	8.5	8.3

1 Per two lambs.
2 On Per lamb.
3 Corrected for the ewe weight changes.
Means in the same line with different superscripts differ statistically ($P < .05$).

Ewe Weight Changes and Feed Efficiency.

The mean values for the two variables are presented in Table 24. Even though the average daily feed intake of most of the experimental groups was higher than that recommended by the NRC (1964), the ewes were not able to maintain constant body weights during the first six weeks of the lactation period. The body weight losses were fairly large for the ewes in the basal and high urea groups. In both

instances the ewes lost most of the weight during the first four weeks and then were not able to gain back to their original weights. A lower feed intake during these early weeks may have been the reason for these losses. The ewes fed the SBM and both low urea rations had lower body losses than the other groups.

All treatments but the basal had higher daily intakes than those recommended by the NRC (1964), and with the exception of the low urea treatment all performed much better than the basal group, indicating that the NRC figure for daily feed intake may be too low for optimal production in ewes with twin lambs.

The mean feed efficiency (FE) values corrected for the body weight changes are presented in Table 24. The ewes in the basal treatment had the lowest FE while those in the SBM treatment were the most efficient in transforming the feed energy into final lamb product. The urea treatments did not differ very much in their FE and the values were slightly lower than those for the SBM treatment.

Blood Hemoglobin.

The mean values for the blood hemoglobin concentration of the ewes in the various treatments, is presented in Table 25. The ewes fed the low urea ration had lower hemoglobin concentrations ($P < .05$) than the ewes in the rest of the experimental treatments. VIRTANEN (1969) reported lower hemoglobin values in milking cows fed purified urea diets than those offered normal diets.

The «F» value for the effect of time on the blood hemoglobin concentration barely reached significance at the five percent level of probability, and the Duncan test did not detect any difference between the samples taken at different times during the lactation period. Because there was no significant interaction between the different treatments and time, the values for the different times were averaged across treatments and they are presented in Table 25. From that Table it seems aparent that there is a clear trend to have lower hemoglobin concentrations as the lactation progresses.

The blood hemoglobin concentration described for the lactating ewes are lower than those previously reported for the growing lambs (Table 7).

Plasma Proteins.

The mean plasma protein concentration values for the ewes in the different treatments are presented in Table 25. The changes in the plasma protein concentration with time are presedted in Table 25.

No differences were observed in the plasma protein concentration among the different treatments. The plasma protein concentration increased with time through the fourth week ($P < .05$) and thereafter remained constant until the sixth week. The lower protein concentration in the blood of the ewes during the early periods of the lactation corresponds with the higher milk production and the higher protein demand for the milk synthesis.

TABLE 25.

Experiment 2. Trial 1. Average Hemoglobin, Plasma Proteins, Blood Ammonia Nitrogen and BUN concentrations of the ewes in the different experimental treatments, and at different times after lambing.

Treatment	Hb gm/100 ml.	Plasma Prot. gm/100 ml.	Blood NH_3 mg./100 ml.	BUN mg./100 ml.
Basal	10.00 b	6.14	.238	7.59
SBM	9.94 b	6.46	.257	15.30 a
Urea	8.86 a	6.41	.250	23.23 b
Urea Adapted	9.38 a, b	6.19	.256	21.84 b
High Urea	10.14 b	6.11	.415 a	49.19 c
Time (weeks)				
0	10.04	5.79	—	19.23
2	10.00	6.13	.291	19.42
4	9.30	6.53	.286	22.43
6	9.23	6.68	.245	25.94
$s_{\bar{x}}$	0.08	0.15	0.06	2.81

Means in the same colum with different superscript differ statistically (P .05).

Blood Ammonia Nitrogen.

The mean blood ammonia nitrogen concentration values for the different experimental treatments are shown in Table 25. The high urea treatment produced a higher blood ammonia concentration ($P < .05$) than the rest of the treatments. All the values are in the high side of the normal range but hey are below the reported toxic levels of 1 to 4 mg. per 100 ml. (CHALUPA, 1968). The high ammonia concentration in the high urea treatment may be indicative of the existence of subclinical toxicity that while it did not seem to have affected the milk production may have been responsible for the large body losses of the ewes in this treatment. Even though the daily nitrogen intake was very different among the other experimental treatments, there were no differences in the concentration of the ammonia in the peripheral blood. This would agree with early reports indicating that changes in the ammonia concentration in the peripheral blood only take place when there is a high rumen ammonia (LEWIS *et al.*, 1957). There were no significant differences in the blood ammonia concentrationa among the samples taken at different times during the lactation.

Blood Urea Nitrogen.

The mean BUN concentration values for the different experimental treatments are presented in Table 25. The ewes being fed the basal ration had the lowest ($P < .05$) BUN concentration. The ewes receiving the SBM ration had lower BUN concentration ($P < .05$), than those fed an isonitrogenous ration supplemented with urea instead of SBM, whether or not the ewes were «adapted» to the urea

feeding. The ewes in the high urea treatment had the highest ($P < .05$) BUN concentration of the different experimental groups. These results would indicate the existence of a close relationship between the BUN concentration and both the intake of nitrogen and the source of nitrogen in the lactating ewes. These results agree well with those reported for the growing lamb experiment (Table 7). The «adapted» ewes performed substantially better than those that were non-adapted, and yet the BUN concentration of both ewes was similar, indicating that the BUN concentration was a poor indicator of the nitrogen retention. The same was true for the growing lambs fed the mineral supplemented diets which improved growth (Table 4) but did not produce any change in the BUN concentration in relation with the urea diets (Table 7).

The BUN concentration of the different ewes increased with time after the second week of the lactation ($P < .05$), which may be a reflexion of a higher feed intake as the lactation progresses.

Trial 2.

Lamb Gains.

The mean values for the ADG and the total lamb production per ewe are presented in Table 26. The ADG of the lambs in the SBM treatment was about 18 percent higher than that of the other experimental groups, but this difference was not statistically significant at the five percent level of probability. Neither the «adaptation» nor the mineral supplementation produced any improvement in the ADG of the lambs in the urea treatments.

Ewe Weight Changes and Feed Efficiency.

The mean values for the ewes body weight changes, the daily feed intake and the feed efficiency for this trial are presented in Table 26.

TABLE 26.

Experiment 2. Trial 2. Average Lamb Gains, Ewe Weight Changes, Daily Feed Intake and Feed Efficiency.

	Treatments				
	SBM	Urea	«Adapted»	Mineral	Mineral «Adapted»
No. of ewes	8	8	7	10	7
Ave. Lamb gain (Kg)	18.5	15.5	14.2	15.0	15.4
ADG (gm)	220	184	171	179	184
Ave. ewe wt. change (kg)	-3.3	-6.6	-2.5	-3.9	-5.5
Ave. daily ewe feed (kg)	2.6	2.5	2.6	2.5	2.6
Total feed/lamb gain	7.6	11.4	10.3	9.7	10.7

All the experimental treatments resulted in body weight losses during the experimental period. The largest losses corresponded to the urea and the «adapted» ewes fed the mineral supplemented diet. The «adaptation» to the urea and the mineral supplementation reduced the losses of the urea fed animals. The SBM treatment resulted in the lowest losses of body weight.

There were no differences in the daily feed intake among the ewes in the different experimental treatments. As in the previous trial the ewes fed the SBM diet had the better FE. Both the adaptation to the urea feeding and the mineral supplementation improved the FE of the urea fed ewes.

Blood Hemoglobin.

The mean blood hemoglobin concentration values for the different treatments are shown in Table 27. There were no statistical differences among the different

TABLE 27

Experiment 2. Trial 2. Average Blood Hemoglobin, Plasma Proteins, Blood Ammonia Nitrogen and BUN concentrations for the ewes in the different treatments and times after lambing.

Treatment	Hb gm/100 ml.	Plasma Prot. gm/100 ml.	Blood NH_3 mg./100 ml.	BUN mg./100 ml.
SBM	9.9	6.27 a	.325 a	56.28 a
Urea	9.7	6.16 a	.431 b	77.06
Urea adapted	9.1	5.80 c	.367 a. b	78.05
Urea + Mineral	9.5	6.04 a, b, c	.403 b	72.84
Urea + Mineral adapted	9.6	5.86 b, c	.448 b	73.02
Time (weeks)				
0	10.59	5.81	.314	40.71
2	10.45	6.15	.410	70.36
4	9.11	6.01	.448	91.97
6	8.22	6.26	398	80.92
$s_{\bar{x}}$	.04	.13	.07	7.4

Means in the same column with different superscripts differ statistically ($P < .05$).

treatments in the hemoglobin concentration. The values are again lower than those described for the growing lambs (Table 7) and similar to those reported for the previous trial (Table 26). Pope *et al.* (1953) have reported lower concentration of the blood hemoglobin for lactating than for pregnant and normal ewes. Swenson (1970) also indicates that lactating cows have low blood hemoglobin concentration.

The hemoglobin concentration decreased with time after lambing ($P < .05$) as can be seen in Table 27.

Plasma Proteins.

The plasma protein means for the different treatments are shown in Table 27. The adapted ewes had lower plasma protein concentration ($P < .05$) than the ewes fed the urea diets without adaptation and lower than the SBM fed animals. The plasma protein concentration increased with time after lambing as can be seen in Table 27 and after six weeks of lactation the values were higher than after lambing. The increase observed in this trial, is smaller than that described for Trial 1. (Table 25).

Blood Ammonia Nitrogen.

The mean blood ammonia nitrogen values for the different treatments are shown in Table 27. The SBM treatment had the lowest blood ammonia concentration ($P < .05$) of the different experimental groups. Neither the «adaptation» nor the mineral supplementation produced any changes in the concentration of the peripheral blood ammonia of the urea fed ewes.

The ammonia concentrations of the ewes in this trial are similar to the values described for the high urea group in Trial 1 (Table 25). These results would indicate a certain response of the blood ammonia concentration to the nitrogen intake when fed at high levels, especially when urea is used as the main nitrogen source. This response does not seem to be as consistent as that observed for the BUN. The presence of high ammonia concentrations in the peripheral blood would indicate high rumen ammonia concentration (Lewis *et al.*, 1957) and also the need for the organism to detoxify extra amounts of ammonia in the liver, that together with the possibility of the ewes suffering a subclinical toxicity may be part of the reason of the poor performance observed in this trial, especially the urea fed groups.

The blood ammonia concentration increased through the first two weeks of the lactation ($P < .05$) and ramained constant thereafter.

Blood Urea Nitrogen.

The mean BUN values for the different treatments are presented in Table 27. As it could be expected from our previous results, both with the lactating ewes and the growing lambs, the BUN concentration of the SBM treatment was lower ($P < .05$) than that of the urea groups. There were no differences in the BUN among the different urea treatments. The BUN values described for this trial are higher than those reported for the Trial 1 (Table 25), indicating the possibility of larger urinary losses (Thorton, 1970a, 1960b, 1970c, 1970d) which would mean a suboptimal nitrogen utilization. This lower nitrogen utilization may have been another reason for the poor performance of the ewes in this trial.

The BUN increased through the fourth week of the lactation and then dropped slightly at the sixth week, but changes were not statistically significant at the five percent level of probability.

Trial 3.

Lamb Gains.

The mean ADG and total lamb produced per ewe are presented in Table 28.

The SBM treatment had higher ADG ($P < .05$) than the other experimental treatments. The SBM fed ewes also produced more lamb per ewe than the urea fed animals. Neither the adaptation to the urea nor the mineral aupplementation had any effect on the growth of the lambs on the urea treatments.

Ewe Weight Changes and Feed Efficiency.

The mean values for these variables are presented in Table 28. The ewes in the SBM treatment had smaller weight losses during the duration of the trial than the ewes in the urea treatments. The adaptation to the urea feeding and the mineral supplementation decreased the weight losses of the urea fed ewes. The body weight losses of the SBM treatment may be considered normal for this type of production, but those of the urea treatments are a little too high and it is felt it will be desirable to have the ewes not lose so much weight during the early lactation (HOGUE, 1968).

TABLE 28.

Experiment 2. Trial 3. Average Lamb Gains, Ewe body weight changes, Feed Intake and Feed efficiency values.

	Treatments			
	SBM	Urea	Adapted	Minerals
No. of ewes	6	10	10	10
Ave. Lamb gain (kg)[1]	22.1	18.1	18.2	18.1
ADG (gm)[2]	263	217	216	214
Ave. ewe wt. change (kg)	-2.8	-7.3	-5.2	-4.8
Ave. daily ewe feed (kg)	2.8	2.8	2.8	2.8
Total feed/lamb gain[3]	6.7	11.1	9.3	9.2

1 Pero two lambs.
2 Per lamb.
3 Corrected for the ewe weght changes.

As reported for the Trial 2 (Table 26) there were no differences in the daily feed intake of the ewes in the various experimental treatments, but here again the SBM fed ewes had a better feed efficiency than the urea fed ewes. The adaptation to the urea feeding and the mineral supplementation improved the feed efficiency of the urea treatments.

Blood Urea Nitrogen.

The mean BUN concentration values are presented in Table 29. As we have been describing all along our different trials, the BUN concentration was lower in the SBM treatments than in the urea fed ewes, however, the differences were not statistically significant at the five percent level of probability. While the BUN values for the SBM Treatment were similar to those reported for Trial 2, (Table 27) those of the urea treatments were much lower than the corresponding values in Trial 2. Since the nitrogen intake was essentially the same in both trials, these results would indicate that the higher energy concentration of the diets on Trial 3, resulted in a better utilization of the nitrogen present in the diet. The results would demonstrate once more the well established fact of the importance of having an adequate carbohydrate source when high urea levels are being used in the rations of ruminants.

TABLE 29

Experiment 2. Trial 3. Average Blood Urea Nitrogen Concentration.

Treatments	BUN (mg/100 ml)
SBM	56.6
Urea	61.0
Urea Adapted	62.3
Urea + Minerals	61.6

Blood Urea Nitrogen and Nitrogen Intake.

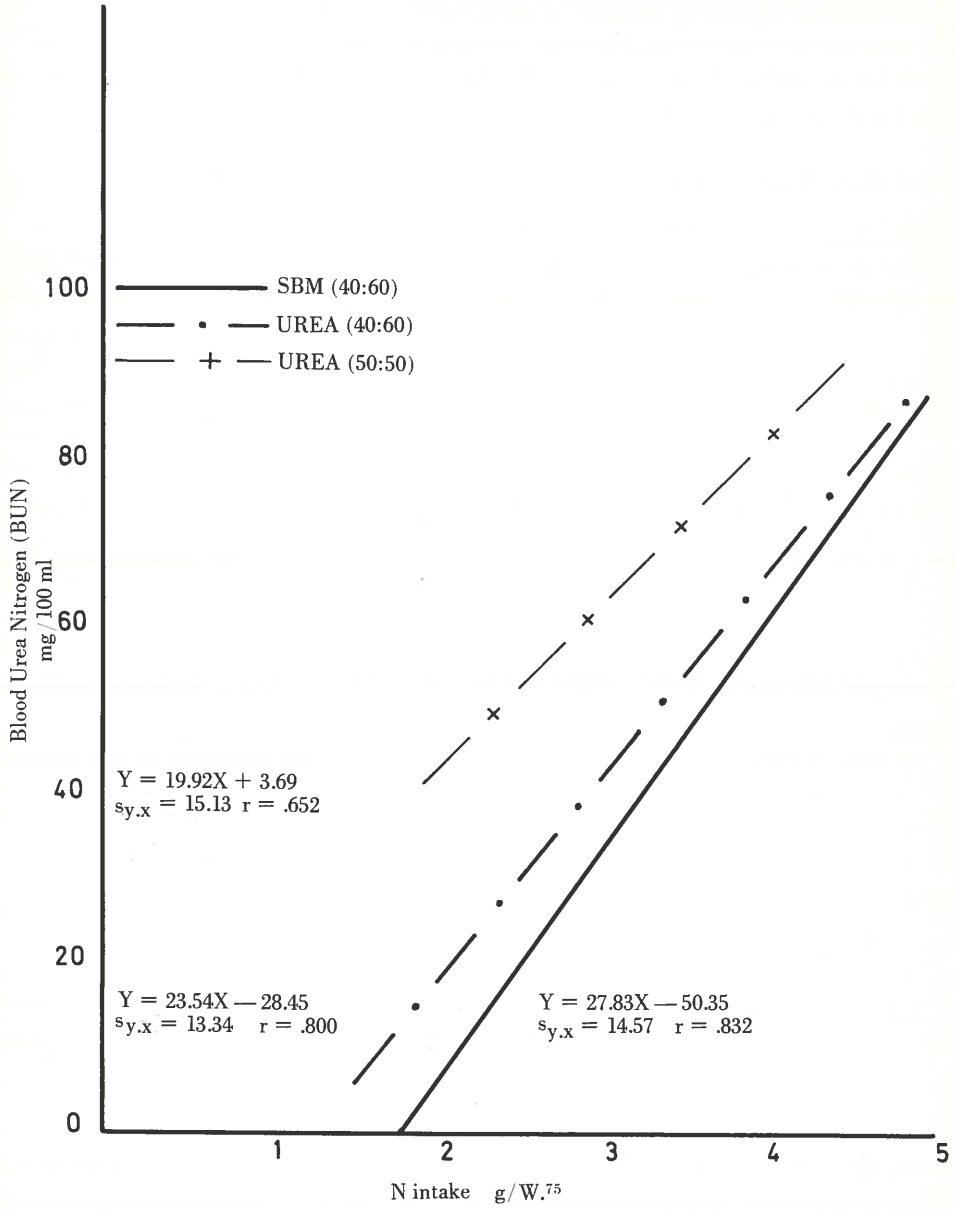
The results presented in this study, both for the growing lambs (Table 7) and the lactating ewes (Tables 25, 27 and 29) clearly indicate that the BUN concentration is closely related to both nitrogen intake and source of nitrogen. We already have discussed the factors that influence the BUN concentration and demonstrated that the most important one is the rumen ammonia formation, which in turn is mainly affected by the nitrogen intake, the type of nitrogen (protein) and the amount of readily available carbohydrate present in the rumen.

PRESTON *et al.* (1965) reported that BUN could be quantified with the protein intake per unit $W.^{75}$, and reported a high correlation (r) of .986 between both factors. The authors indicated that limited energy and different proteins could upset the relationship presented. No information has been found in the literature indicating how these factors would modify the mentioned relationship.

Since in our lactating ewe experiments we used different nitrogen sources and different energy concentrations in our experimental rations, it was decided to combine the data of the different trials and try to quantify the changes in BUN with the nitrogen intake expressed in grams per $W.^{75}$ for the different nitrogen sources fed at the two levels of energy concentration. First of all the observations were grouped according to the source of nitrogen (SBM or urea) and then divided within

each group according to the energy concentration of the rations (different hay: grain ratios). Simple linear regression analysis was used and the resulting equations are shown in Figure 2.

Figure 2. Regression equations of N intake on BUN for diets supplemented with Urea or SBM fed to lactating ewes



There was no significant regression of the BUN on the intake of nitrogen for the ewes fed the 50 : 50 hay: grain ratio supplemented with SBM. The energy concentration of the diet was very important for the nitrogen utilization in the case of the urea fed animals. A highly significant ($P < .005$) and positive linear relationship between the BUN and the nitrogen intake is described for each of the two energy levels. The equations describing the relationship at the two energy levels had a significantly different intercept ($P < .005$), but their slopes (ß) were not found to be statistically different at the five percent level, however, the regression line for the low energy diets (50 : 50) was displaced to the left of the line for the 40 : 60 rations. This means that at similar nitrogen intakes, the BUN concentration of the animals on the low energy ration was considerable higher than in the high energy rations, and consequently the urinary losses will also be higher and therefore a poorer nitrogen utilization may be expected under the low energy condicions.

There was also a positive and linear relationship ($P < .05$) between the BUN concentration and the nitrogen intake in the ewes fed the SBM rations with a 40 : 60 hay : grain ratio. The regression describing this relationship was displaced to the right of those for the urea fed animals. This regression had a different intercept (α) than those for the urea treated animals ($P < .005$), but its slope (ß) was not different at the five percent level; however, when the ten percent level was used the slope resulted to be different from that of the regression for the urea fed ewes receiving the 50 : 50 ration.

These results will agree with the reports indicating that the overall nitrogen intake is not the main factor in controlling the BUN concentration and that the formation of ammonia in the rumen seems to be more important in this respect (Lewis, 1957; Abou Akkada and El-Sayed Osman, 1967).

These results would indicate that if the BUN is to be used as an index of the nitrogen status or the nitrogen utilization by ruminants, more information is needed in order to determine the effect that different protein sources may have on the BUN concentration. It will also be desirable to try to define the optimal energy : protein ratio, especially in the case of NPN rations, for the different sources and levels of nitrogen in the ration of the ruminants.

The suboptimal performance of the ewes fed the 50 : 50 rations could be explained due to a deficiency in energy in the case of the urea fed ewes and to a marginal level in the case of the SBM treatment, which in both cases may have prevented the establishement of an optimal protein synthesis in the rumen. In the case of the urea fed animals, large amounts of ammonia may have been absorbed from the rumen into the portal systen and taken into the liver where needed energy had to be used in the unproductive process of detoxifying the extra amount of ammonia present.

Limitations of the NRC Requirements for Lactating Ewes.

The 1964 edition of the NRC requirement for sheep does not yet differentiate between the needs of the lactating ewes suckling one or more lambs, and recommends a requirement for the lactation period regardless of the number of lambs suckled. Reports by GARDNER and HOGUE (1963, 1964, 1966, 1967) and HOGUE (1968), indicated that the energy and protein requirements recommended by the NRC for lactating ewes were inadeauate for ewes suckling twin lambs. In our study we have shown how the milk production of ewes could be improved by increasing the protein content of the diet from eight percent to about twelve percent (Trial 1), this increased milk yield resulted in a higher lamb production per ewe. These results will agree with those of POPE *et al.* (1952, 1953) for ewes with single lambs and with GARDNER and HOGUE (1967) for both single and twin lambs, and will point out once more that the eight percent recommended by the NRC is too low for optimal production. The slight increase in lamb growth observed when the protein content was increased from 12 percent to about 18 percent (Trials 1 and 3), would indicate that the protein requirement may be higher than the 12 percent recommended by HOGUE (1968), when poor quality forages such as those employed here are fed as the main roughage.

Another aspect that it is felt deserves some further study is that of establishing adequate energy requirements for the lactating ewes with twin lambs. The 1964 NRC requirements, recommends the feeding of about five MegCal of ME per day during the first eight weeks of the lactation. HOGUE (1968) goes on to recommend a daily requirement of about 6.9 MegCal of ME/day. The estimated average daily intake of ME in our experiment was about 6.0 MegCal of ME/day for the 50 : 50 rations supplemented with SMB; and about 5.7 MegCal of ME/day for the urea fed animals. The ewes receiving the 40 : 60 rations were receiving about 6.5 MegCal of ME/day in the SBM treatments and about 6.6 MegCal of ME/day for the urea fed ewes. The results of Trial 3 indicate that the ewes fed the 40 : 60 rations performed better across the different treatments than those in Trial 2, that were fed the 50 : 50 ration. This improvement was larger for the urea fed ewes and we already have discussed the possible reasons for this improved performance.

These results would tend to indicate that for lactating ewes with twin lambs fed rations similar to those used in our work (use of poor quality forages), a hay : grain ratio of 40 : 60 may be more adequate than the more widely used of 50 : 50 in the practical rations.

In general our results would agree better with HOGUE's recommendations (1968) than with the old NRC requirements. The new NRC table of requirements for sheep (1971), has differentiated for the first time between lactating ewes suckling single and twin lambs and recommends the feeding of 6.1 MegCal of ME/day to an average 60 kg. lactating ewe with twin lambs, which will be in good agreement with our results.

Urea versus SBM for Lactating Ewes.

The results presented in this work (Tables 24, 26 and 28) would indicate that urea is inferior as a source of nitrogen to SBM in rations for lactating ewes containing more than twelve percent protein equivalent. This agrees very well with our results for the growing lambs (Table 4). The results will also fall in line with those of the reports discussed in the literature review indicating that increased levels of urea in the rations for dairy cows reduced the milk yield.

The «adaptation» to the urea feeding improved the milk production, and therefore the lamb growth, in the animals fed the urea diets with a protein equivalent of about twelve percent to values similar to those obtained with an isonitrogenous SBM diet. There was not such an «adaptation» response when the protein equivalent of the ration was about 18 percent. We already have indicated that at the high urea levels there was a high blood ammonia nitrogen (Table 27) which may be responsible for a subclinical toxicity of the ewes on these high urea rations. The urea levels used in this high urea diets were higher than the recommended safety level of about .3 gm. per kg. of body weight (ARMSTRONG and TRINDER, 1968), and our ewes were receiving about 1.5 gm. per kg. body weight.

Both the «adaptation» and the mineral supplementation of the urea diets resulted in general in decreased body weight losses of the ewes and in a better feed efficiency, while they did not have any effect on the lamb growth, when the high urea rations were fed to the lactating ewes.

In summary it can be said, once more, that the substitution of urea for SBM in the rations for the lactating ewes resulted in a decreased productivity, measured as lamb produced per ewe, when the protein equivalent of the rations was higher than twelve percent. At the twelve percent protein equivalent the urea fed ewes performed as well as those receiving the SBM ration.

CONCLUSIONS

As a result of these experiments it can be concluded that:

1) The protein requirement of the lactating ewes suckling twin lambs should be higher than the eight percent figure recommended by the NRC, and in order to obtain maximum productivity of the ewes, values higher than the twelve percent may be required.

2) When poor quality forages are used in the rations for lactating ewes suckling twin lambs, a hay to grain ratio of 40 : 60 seems to yield better production than the more conventional 50 : 50 rations.

3) Feeding lactating ewes with rations supplemented with urea at protein equivalent higher than twelve percent decreased the production of the ewes fed similar rations containing SBM instead of the urea.

4) The BUN of the lactating ewes in our experiment was definitely influenced by the source of nitrogen and the energy concentration of the ration besides the overall nitrogen intake.

5) The blood hemoglobin concentration of the lactating ewes under our experimental conditions, was lower than the reported values for the growing lambs; the average value was 9.6 mg./100 ml.

RESUMEN

En el presente trabajo se ha estudiado el valor de la urea como principal fuente de nitrógeno para corderos en crecimiento y ovejas lactantes, para lo cual se realizaron dos series de experimentos. En la primera (Serie 1) se utilizaron corderos en las últimas fases del crecimiento y en la segunda (Serie 2) se emplearon ovejas lactantes, amamantando corderos gemelos.

En la *primera serie* se añadieron diversos elementos minerales (K, S, Fe, Cu y Mn) a raciones a base de urea para igualar su contenido en tales elementos al de raciones isonitrogénicas a base de harina de soja. Los animales experimentales, 144 corderos en pruebas de crecimiento y metabolismo, recibieron ambos tipos de raciones (soja y urea) a dos niveles de proteína bruta (12 y 16 %).

La adición de los elementos minerales mejoró ($P < 0,05$) la ganancia en peso de los animales recibiendo urea, tanto al nivel del 12 % como al del 16 % de proteína bruta. Los niveles de nitrógeno uréico en plasma fueron superiores ($P < 0,05$) en los corderos que recibieron las raciones con urea, respecto a los alimentados con harina de soja. La adición de minerales no produjo ningún cambio en los valores de nitrógeno uréico plásmático en los animales que recibieron las raciones con urea; sin embargo mejoró el nivel de ingestión y el índice de transformación, medidos durante un período de 100 días.

En el experimento de metabolismo, se utilizaron 20 corderos provistos de una fístula ruminal, divididos en 5 grupos, que recibieron los siguientes tratamientos: ración basal con un 12 % de proteína bruta, con urea como principal fuente de nitrógeno; ración basal suplementada con todos los elementos minerales mencionados (K, S, Fe, Cu y Mn); basal más K; basal más S; basal más Cu + Mn. Los animales permanecieron en los diferentes tratamientos experimentales durante un período de 30 días, al cabo de los cuales no se encontraron diferencias ni en la concentración de ácidos grasos volátiles ni en la de nitrógeno uréico en la sangre. Sin embargo la adición a la ración basal de Cu + Mn redujo significativamente ($P < 0,05$) la concentración de amoníaco en el rumen a los 15 días, desapareciendo esta diferencia a los 30 días. La adición de Cu + Mn mejoró la retención de nitrógeno al cabo de 30 días ($P < 0,05$), pero tal efecto no se manifestó a los 15 días.

En la *segunda serie*, un grupo de 102 ovejas lactantes amamantando corderos gemelos, fueron alimentadas individualmente durante las primeras seis semanas de la lactación con raciones conteniendo tres niveles de proteína bruta (8, 12 y 18 %) y dos proporciones de heno a concentrado (40 : 60 y 50 : 50). Las raciones contenían soja o urea como principal fuente de nitrógeno. En esta Serie se realizaron tres experimentos en los que se estudió: a) el efecto de la adición de elementos minerales (K, S, Fe, Cu y Mn) a raciones a base de urea hasta igualar el contenido en tales elementos al de raciones isonitrogénicas a base de soja y b) el «efecto de adaptación» a la alimentación con raciones conteniendo urea.

La producción de leche y las ganancias en peso de los corderos aumentaron significativamente ($P < 0,05$) al incrementarse el contenido en proteína bruta de una ración basal de heno y maíz (40 : 60) del 8 % al 12 % mediante la adición de harina de soja. El «efecto de adaptación» a las raciones de urea mejoró el crecimiento de los corderos y la producción de leche ($P < 0,05$) de las ovejas que recibieron tales raciones. Los niveles de nitrógeno uréico en la sangre de estas ovejas fueron más elevados ($P < 0,05$) que los correspondientes a las ovejas que consumían raciones isonitrogénicas a base de soja.

Ni la adición de los elementos minerales ni el «efecto de adaptación», mejoraron las ganancias de peso de los corderos amamantados por ovejas que recibían una ración conteniendo el 18 % de proteína bruta, con una relación heno: concentrado de 50 : 50. Por otra parte los incrementos de peso de los corderos cuyas madres recibieron raciones de soja (18 % P. B. y 50 : 50) fueron ligeramente superiores a los de los corderos de ovejas alimentadas con urea, aunque stas diferencias no fueron estadísticamente significativas. Tanto la adición de elementos minerales, como el «efecto de adaptación», mejoraron el índice de transformación en las ovejas consumiendo las dietas a base de urea y redujeron las pérdidas de peso sufridas por las ovejas durante las primeras seis semanas de lactación.

Los niveles de hemoglobina en sangre, correspondientes a las ovejas bajo los distintos tratamientos experimentales, disminuyeron significativamente a lo largo de las seis primeras semanas de la lactación. Los valores obtenidos resultaron inferiores a los normalmente señalados para corderos en crecimiento.

Finalmente se señala la existencia de una relación lineal positiva entre la concentración de nitrógeno uréico en sangre (mg/100 ml) y la cantidad de nitrógeno ingerida ($gm/Kg^{0,75}$), que resultó ser estadísticamente significativa ($P < 0,005$). Las rectas de regresión correspondientes a los animales que recibieron urea (40 : 60 y 50 : 50) tienen una pendiente (b) similar a la correspondiente a los animales que recibieron soja (40 : 60), pero las intersecciones (a) resultaron ser diferentes.

RESUME

Dans ce travail on a étudié la valeur de l'urée comme principale source de nitrogène (ou azote) pour des agneaux en accroissement et des brebis en période de lactation; à cet effect, on a fait deux séries d'expériences. Dans la première (Série 1) on utilisa des agneaux dans leurs dernières phases d'accroissement, et dans la deuxième (Série 2) on utilisa des brebis en période de lactation, qui allaitaient des agneauc jumeaux.

Dans la *première série* on ajouta divers éléments minéraux (K, S, Fe, Cu et Mn) à des rations qui contenaient de l'urée, à fin d'égaler la teneur de ces éléments à celle des rations isonitrogénées qui contenaient de la farine de soja. Les animaux expérimentaux, 144 agneaux en accroissement et en métabolisme, reçurent les deux types de rations (soja et urée) à deux niveaux différents de protéine crue (12 % et 16 %).

L'addition des éléments minéreaux augmenta ($P < 0,05$) le poids des animaux qui avaient reçu de l'urée au niveau du 12 % de protéine crue, à l'égal que celui des animaux qui avaient reçu de l'urée au niveau du 16 % de cette même protéine. Les niveaux de nitrogène uréique dans le plasma furent supérieurs ($P < 0,05$) dans les agneaux qui avaient reçu les rations avec de l'urée, en rapport avec les agneaux alimentés avec de la farine de soja. L'addition des minéraux ne produisit aucun changement dans les valeurs de nitrogène uréique plasmatique chez les animaux qui avaient reçu les rations avec de l'urée, en rapport avec les agneaux alimentés avec de la farine de soja. L' addition des minéraux ne produisit aucun changement dans les valeurs de nitrogène uréique plasmatique chez les animaux qui avaient reçu les rations avec de l'urée; cependant, elle augmenta le niveau d'ingestion et l'index de transformation, déterminés pendant une période de 100 jours.

Dans l'expérience de métabolisme on utilisa 20 agneaux munis d'une fistule ruminante, distribués en cinq groupes, qui reçurent les traitements suivants: ration basale contenant un 12 % de protéine crue, avec de l'urée comme source principale de nitrogène; ration basale à laquelle on ajouta tous les éléments minéraux indiqués (K, S, Fe, Cu et Mn); ration basale + K; ration basale + S; ration basale + Cu + + mn). Les animaux furent traités expérimentalement pendant 30 jours, au bout desquels on ne trouva des différences ni dans la concentration d'ácides gras valatils dans le rumen, ni dans celle de nitrogène uréique dans le sang. Cependant, l'addition de Cu + Mn à la ration basale, réduisit significativement ($P < 0,05$), mais cet effet ne se manifesta pas après les 15 premiers jours.

Dans la *dexuième série*, un groupe de 102 brebis en période de lactation, qui allaitaient des agneaux jumeaux, furent alimentées individuellement pendant les six premières semaines de lactation avec des rations contenant trois niveaux de protéine crue (8 %, 12 % et 18 %) et deuz proportions de foin concentré (40 : 60 et 50 : 50). Les rations contenaient du soja ou de l'urée comme principale source de nitrogène. Dans cette série on réalisa trois expériences dans lesquelles en a étudié: a) l'effect de l'addition d'éléments minéraux (K, S, Fe, Cu et Mn) à des rations contenant de l'urée, jusqu'à égaler la teneur des susdits éléments minéraux avec celle des rations isonitrogénées contenant du soja; b) l'effet d'adaptation à l'alimentation avec des rations contenant de l'urée.

La production de lait et les gains en poids des agneaux augmentèrent significativement ($P < 0,05$) quand on augmenta la teneur en protéine crue d'une ration basale de foin et de mais (40 : 60) du 8 au 12 %, moyennant l'addition de farine de soja. L'effet d'adaptation aux rations d'urée augmenta l'accroissement des agneaux et la production de lait ($P < 0,05$) des brebis qui avaient reçu les susdites rations. Les niveaux de nitrogène uréique dans le sang de ces brebis furent plus élevés ($P < 0,05$) que ceux des brebis qui recevaient des rations isonitrogénée contenant du soja.

Ni l'addition des éléments minéraux ni l'effect d'adaptation augmenterent les gains en poids des agneaux allaités par des brebis qui recevaient une ration qui contenait un 18 % de protéine crue et une proportion de foin concentré de 50 : 50. D'autre part, les gains ou augmentations en poids des agneaux dont les mères avaient reçu des rations de soja (18 % de protéine crue et 50 : 50 de foin concentré) furent légèrement supérieurs à ceux des agneaux dont les mères aveient reçu des rations d'urée, quoique ces différences n'étaient pas significatives. L'addition d'éléments minéraux, ainsi que l'effet d'adaptation augementèrent l'index de transformation chez les brebis alimentées avec des rations contenant de l'urée, et réduisirent les pertes subies par les brebis pendant les six premières semaines en période de lactation.

Les niveaux d'hémoglobine du sang correspondant aux brebis soumises aux différents traitments expérimentaux diminuèrent significantivement pendant les six premières semaines de la période de lactation. Les valeurs obtenues furent inférieures à celles normalement indiquées pour des agneaux en accroissement.

Finalement, on indique l'existence d'une proportion linéaire positive entre la concentration de nitrogène uréique dans du sang (mg/100 ml) et la quantité de nitrogène ingérée (gramme/Kg 0,75) qui résulta être statistiquement significative ($P < 0,005$). Les lignes droites de regression correspondantes aux animaux qui reçurent de l'urée (40 : 60 et 50 : 50) ont une déclination ou pente (b) similaire à celle qui correspond aux animaux qui reçurent du soja (40 : 60), mais les intersections (a) sont différentes.

SUMMARY

The value of urea (U) as the main source of nitrogen for growing lambs and lactating ewes was studied in this work. Two different experiments were conducted, one with growing finishing lambs (Experiment 1), and the second one with lactating ewes suckling twin lambs (Experiment 2).

In Experiment 1, minerals (K, S, Fe, Cu and Mn) were added to urea diets to equate them to isonitrogenous Soybean Meal (SBM) diets. Growth and metabolism trials were conducted with lambs fed these diets at two different crude protein (CP) levels (12 and 16 %). In a growing trial with 6 groups of 24 lambs, the addition of minerals increased ($P < .05$) the weight gains of the lambs fed either a 12 or 16 % CP diets over 100 days. The average Plasma Urea Nitrogen (PUN) was higher ($P < .05$) in the lambs fed the urea diets than in those fed the SBM diets. The addition of minerals, did not produce any change in the PUN of the urea fed animals. The mineral additions improved the daily feed intake and the feed efficiency (FE) of the urea fed lambs. Fice groups of 4 sheep with rumen fistulae, were then fed the 12 % CP urea diet and diets supplemented with either all minerals (M), or K, or S, or Cu + Mn, in a metabolism trial for 30 days. No differences were found in the rumen VFA's or Blood Urea Nitrogen (BUN) concentrations; however, the addition of Cu + Mn to the urea diet, significantly reduced ($P < .05$) the rumen ammonia concentration after 15 days on the diets. No differences were observed after 30 days. The addition of Cu + Mn, increased the nitrogen retention of the urea fed animals after 30 days on the diets ($P < .05$), but it did not produce any changes in the nitrogen retained after 15 days.

In Experiment 2, lactating ewes suckling twin lambs, were individually fed during the first six weeks of the lactation period different rations containing 3 CP levels (8, 12 and 18 %) and 2 hay: grain ratios (40 : 60 and 50 : 50) with urea or SBM as the main source of nitrogen. The addition of minerals (K, S, Fe, Cu and Mn) to urea diets to equate them to isonitrogenous SBM diets, and the «adaptation» of the ewes to the urea feeding were studied in three different trials, with the following results:

Increasing the CP content of a 40 : 60 hay: corn basal (B) diet from 8 to 12 % by adding SBM increased the milk yield and the total lamb gains ($P < .05$). The «adaptation» to the urea feeding increased the milk yield and the lamb gains of the urea fed ewes ($P < .05$). The ewes fed the urea diets had higher ($P < .05$) BUN than those fed isonitrogenous SBM diet. Neither the mineral additions nor the «adaptation» to the urea improved the lamb gains of ewes fed a 50 : 50 hay grain ration at 18 % CP. The lamb gains of the SBM fed ewes were higher than those fed the urea rations, but these differences were not statistically significant. The urea fed ewes had higher ($P < .05$) BUN concentration than those fed isonitrogenous SBM diet. The addition of minerals and the «adaptation» to the urea improved the FE of the urea fed ewes and reduced the body weight losses during the first six weeks of the lactation.

Ewes fed a 40 : 60 hay: grain ration supplemented with SBM, had higher lamb gains ($P < .05$) than those fed an isonitrogenous urea diet. Neither the «adaptation» nor the mineral additions increased the lamb gains of the urea fed ewes. The urea fed ewes had higher BUN concentrations than those fed the SBM supplemented ration, but the difference was not statistically significant. Both the «adaptation» to the urea feeding and the addition of the minerals improved the FE of the urea fed ewes and reduced the body weight losses of the ewes during the first six weeks of the lactation period.

The blood hemoglobin of the ewes in the different trials and treatments decreased ($P < .05$) through the first six weeks of the lactation and the values were lower than those described for the growing lambs.

A highly significant ($P < .005$) positive linear relationship between BUN (mg./100 ml.) and nitrogen intake (gm/W.^{75}) is described for the lactating ewes. Three regression equations having similar slopes (ß), but different intercepts (α) are described for the SBM (40 : 60 ration) and the urea diets (both 40 : 60 and 50 : 60). The two regression equations described for the urea fed ewes were displaced to the left of the SBM regression line.

ACKNOWLEDGEMENTS

The author wishes to express his gratitude to Dr. Douglas E. Hogue for acting as Chairman of his Graduate Committee and for the confidence he always deposited on thim.

It has been a privilege to have Dr. W. G. Pond and Dr. M. J. Wright as members of his Graduate Committee.

The author is particularly grateful to Mr. G. L. Hunt for his enthusiasm in taking care of the management of the experimental animals. His experience and interest, made working with him a valuable learning experience.

The cooperation of Earl Walker with most of the laboratory work and the help of Alan Sutton in the performance of the surgical procedures are gratefully appreciated. Thanks are also extended to Dr. P. D. Miller for his help with some of the statistical analysis, and to Linda Lemka, for her cooperation and efficiency in typing this thesis.

The author wishes to express his gratitude to Cornell University for the assistantship granted to him, and also to the sheep division of the Animal Science Department for providing him with financial aid to be able to finish his Graduate program. Thanks are also given to the Fulbright Commission for the Travel Grant given to him.

BIBLIOGRAPHY

ABOU AKKADA, K. A., and OSMAN, H. E. S. 1967. The use of ruminal ammonia and blood urea as an index of the nutritive value of protein in some foodstuffs. *J. Agric. Sci.*, **69:** 25.

ALBERT, W. W., U. S. GARRIGUS, R. M. FORBES, and G. W. NORTON. 1956. The sulfur requirement of growing-fattening lambs in terms of methionine, sodium sulfate and elemental sulfur. *J. Animal Sci.*, **15:** 559.

ALLAWAY, W. H. 1969. Trends in the mineral composition of feeds. *Proc. Cornell Nutrition Conference for Feed Manufacturers.*

ANDERSON, C. M. 1956. The metabolism of sulfur in the rumen of the sheep. New Zealand *J. Sci. Technol. Sec. A.*, **37:** 379.

ARCHIBALD, J. G. 1943. Feeding urea to dairy cows. *Mass. Agric. Exp. Sta. Bull.* 406.

ARIAS, C., W. BURROUGHS, P. GERLAUGH and R. M. BETHKE. 1951. The influence of different amounts and sources of energy upon «in vitro» urea utilization by rumen microorganisms. *J. Animal Sci.*, **10:** 683.

ARMSTRONG, D. G. and N. TRINDER. 1966. The use of urea and other Non-Protein Nitrogenous substances in rations for ruminants. The Journal of the University of Newcastle Upon Tyne. *Agricultural Society*. **20**:22.

ASSOCIATION OF OFFICIAL AGRICULTURAL CHEMISTS. 1945. *Official and Tentative Methods of Analysis*. 6th ed. Washington, D. C. AOAC.

BALCH, C. C. and R. C. CAMPLING. 1961. Utilization of urea by milking cows. *J. Dairy Res.* **28:** 157.

BARTH, K. M., G. A. MCLAREN and G. C. ANDERSON. 1961. Relationship between microbial protein synthesis and the adaptation response. *J. Animal Sci.*, **20:** 924 (Abstr.)

BELASCO, I. J. 1956. The role of carbohydrates in urea utilization, cellulose digestion and fatty acid formation. *J. Animal Sci.*, **15:** 496.

BELL, M. C., W. D. GALLUP and C. K. WHITEHAIR. 1953. Value of urea nitrogen in rations containing different carbohydrates feeds. *J. Animal Sci.*, **12:** 786.

BLAXTER, K. L. 1968. The animal harvest. *Science Journal*, **4**: 53.

BLOCK, R. J., J. A. STEKOL and J. K. LOOSLI. 1951. Synthesis of sul*fur amino acids from* inorganic sulfate by ruminants. II. Synthesis of cystine and methionine from sodium sulfate by the got and by the microorganisms of the rumen of the ewe. Archives of *Biochemistry and Biophysics*. **33:** 353.

BOSMAN, S. M. 1966. Sulphides in rumen liquor of cattle in relation to the feed and its possible influence on copper metabolism. *Inst. Biol. Scheik. Onderzoik. Landbouwgewasen. Wageningen, Medded* No. 314-32.

BRIGGS, M. H. 1967. *Urea as a Protein Supplement*. Briggs, M. H. ed. Pergamon Press, Edinburgh.

BRYANT, M. P. and I. M. ROBINSON. 1963. Apparent incorporation of ammonia and amino acid carbon during growth of selected species of ruminal bacteria. *J. Dairy Sci.*, **46:** 150.

BULL, L. S. 1969. Effect of acetic acid on energy metabolism and body composition of sheep. PhD. Thesis. Cornell University.

BUNN, C. R. and G. MATRONE. 1968. Dietary factors affecting utilization of urea nitrogen by sheep in purified diets. *J. Nutrition*. **95:** 122.

BURROUGHS, W., P. GERLAUGH and R. M. BETHKE. 1950. The influence of alfalfa hay and fractions of alfalfa hay upon the digestion of ground corncobs. *J. Animal Sci.*, **9:** 207.

BURROUGHS, W., C. ARIAS, P. DE PAUL, P. GERLAUGH and R. M. BETHKE. 1951. «In vitro» observations upon the nature of protein influences upon urea utilization by rumen microorganisms. *J. Animal Sci.*, **10:** 672.

BURTON, J. H. 1967. The effects of age and weight on the body composition of sheep. M. S. Thesis. Cornell University.

CAFFREY, P. J. and G. S. SMITH. 1964. Retention by sheep of NH_4-N infused intravenously for several weeks. *J. Animal Sci.*, **23:** 1.208 (Abstr.).

CAFFREY, P. J., E. E. HATFIELD, H. W. Norton and U. S. Garrigus. 1967. Nitrogen metabolism in the ovine. I. Adjustment to a urea-rich diet. *J. Animal Sci.*, **26:** 595.

CAMPBELL, L. D. and W. K. ROBERTS, 1965. The requirements and role of potassium in ovine nutrition. *Canada J. Animal Sci.*, **45:** 147.

CHALUPA, W., J. L. EVANS and M. C. STILLIONS. 1964. Metabolic aspects of urea utilization by ruminant animals. *J. Nutrition*. **84:** 77.

CHALUPA, W. 1968. Problems in feeding urea to ruminants. *J. Animal Sci.*, **27:** 207.

CHALUPA, W., J. CLARK, P. OPLIGER and R. LAWKER. 1970a. Ammonia metabolism in rumen bacteria and mucosa from sheep fed soy protein or urea. *J. Nutrition*. **100:** 161.

CHALUPA, W., J. CLARK, P. OPLIGER and R. LAWKER. 1970b. Detoxication of ammonia in sheep fed soy protein or urea. *J. Nutrition*. **100:** 170.

CHANEY, A. L. and E. P. MARBACH. 1962. Modified reagents for determination of urea and ammonia. *Clin. Chem.* **8:** 130.

CLARK, J. L. 1969. Evaluation of nitrate, urea and minerals in ruminant rations. Dissertation Abstracts (B), 29, 2.690B.

CLIFFORD, A. J. and A. D. Tillman. 1968. Urea and isolated soybean protein in sheep purified diets. *J. Animal Sci.*, **27:** 484.

COCIMANO, M. R. and R. A. LENG. 1967. Metabolism of urea in sheep. Brit. *J. Nutrition*. **21:** 353.

COOMBE, J. B. and R. R. CHRISTIAN. 1969. The effect of urea on the utilization of ground pelleted roughages by penned sheep. II. Utilization of organic matter, nitrogen and minerals. *J. Agric. Sci.*, **72:** 261.

COULOMBE, J. J. and J. FAREAU. 1963. A new simple semi-micro method for colorimetric determination of urea. *Clin. Chem.*, **9:** 102.

CRAMPTON, E. W. 1964. Nutrient-to-calorie ratios in applied nutriton. *J. Nutrition*. **82:** 353.

DAVIS, R. F., C. WILLIAMS and J. K. LOOSLI. 1954. Studies on the nitrogen sulfur ratios in feeds for dairy cows. *J. Dairy Sci.*, **37:** 813.

DEIF, H. I., A. R. ABOU AKKADA und K. EL-SHAZLY. 1970. A note on the utilization of urea nitrogen by sheep. *Animal Production*. **12:** 339.

DINNING, J., H. M. BRIGGS, W. D. GALLUP, H. W. ORR and R. BULLER. 1948. Effect of orally administered urea on the ammonia and urea concentration in the blood of cattle and sheep. Observations on blood ammonia levels associated with symptoms of alkalosis. *Am. J. Physiol.* **153:** 41.

DRAPER, N. R. and H. SMITH. 1966. *Applied regression analysis*. John Wiley and Sons, Inc., New York.

ELLIS, W. C., and W. H. PFANDER. 1958. The influence of varied cellulose and nitrogen levels upon ration digestibility and nitrogen balance of lambs fed semipurified diets. *J. Nutrition*. **65:** 235.

EMERY, R. S., C. K. SMITH and L. FAITO. 1957a. Utilization of inorganic sulfate by rumen microorganisms. II. The ability of single strains of rumen bacteria to utilize inorganic sulphate. *Applied Microbiology*. **5:** 363.

EMERY, R. S., C. K. SMITK and C. F. HUFFMAN. 1957b. Utilization of inorganic sulfate by rumen microorganisms. I. Incorporation of inorganic sulfate into amino acids. *Applied Microbiology*. **5:** 360.

ERWIN, E. S., G. J. MARIO and E. M. EMERY. 1961. Volatile Fatty Acid. analysis of blood and rumen fluid by gas chromatography. *J. Dairy Sci.*, **44:** 1.768.

FLATT, W. P., P. W. MOE, R. R. OLTJEN, P. A. PUTMAN and N. W. HOOVEN, Jr. 1969. Energy metabolism studies with dairy cows receiving purified diets. in *Energy Metabolism of Farm Animals*. Edt. by K. L. Blaxter, J. Kielanowski and G. Thorbek. Oriel Press Ltd. New Castle upon Tyne.

FOLIN, O., and H. WU. 1919. A system of blood analysis. *J. Biol. Chem.* **38:** 81.

FOLIN, O., and H. WU. 1920. A simplified and improved method for determination of sugar. *J. Biol. Chem.* **41:** 367.

FONTENOT, J. P., W. D. GALLUPAND A. B. NELSON. 1955. Effect of added carbohydrate on the utilization by steers of nitrogen in wintering rations. J. Animal Sci., **14:** 807.

GALL, L. S., W. E. THOMAS, J. K. LOOSLI and C. N. HUHTANEM. 1951. The effect of purified diets upon rumen flora. *J. Nutrition*. **44:** 113.

GARDNER, R. W. and D. E. HOGUE. 1963. Studies on the TDN requirement of pregnant and lactating ewes. *J. Animal Sci.*, **22:** 410.

GARDNER, R. W. and D. E. HOGUE. 1964. Effects of energy intake and number of lambs suckled on milk yield, milk composition and energetic efficiency of lactating ewes. *J. Animal Sci.*, **23:** 935.

GARDNER, R. W. and D. E. HOGUE. 1966. Milk production, milk composition and energetic efficiency of Hampshire and Corriedale ewes fed to maintain body weight. *J. Animal Sci.*, **25:** 789.

GARRIGUS, U. D. 1968. Less expensive proteins (Nitrogen) for sheep diets. Proc. Symposium on Sheep Nutrition and Feeding. Iowa State University.

GORNALL, A. G., C. J. BARDAWILL and M. M. DAVID. 1949. Determination of serum proteins by means of the biuret reaction. *J. Biol. Chem.* **117:** 751.

GOSSET, J. W. and J. K. RIGGS. 1956. The effects of feeding dehydrated alfalfa leaf meal and trace minerals to growing beef calves fed poor quality prairie hay. *J. Animal Sci.*, **15:** 840.

GOSSET, W. H., T. W. PERRY, M. T. MOHLER, M. P. Plumlee and W. M. Beeson. 1962. Value

of supplemental lysine, methionine, methionine analogue and trace minerals on high urea fattening rations for beef steers. *J. Animal Sci.*, **21:** 248.
GREBING, S. E., D. P. HUTCHESON and R. L. PRESTON. 1970. Early reduction in urinary-N by Diethylstilbestrol in lambs. *J. Animal Sci.*, **31:** 763.
HALE, W. H. and U. S. GARRIGUS. 1953. Synthesis of cystine in wool from elemental sulfur and sulfate sulfur. *J. Animal Sci.*, **12:** 492.
HALVERSON, A. W., G. D. WILLIAMS and G. D. PAULSON. 1968. Aspects of sulfate utilization by the microorganisms of the ovine rumen. *J. Nutrition.* **95:** 363.
HAMILTON, T. S., W. B. ROBINSON and B. C. JOHSON. 1948. Further Comparisons of the utilization of nitrogen of urea with that of some feed proteins by sheep. *J. Animal Sci.*, **7:** 26.
HEAD, M. J. 1953. The effect of quality and quantity of carbohydrate and protein in the ration of the sheep on the digestibility of cellulose and other constituents of the ration, with a note on the effect of adding vitamins of the B-complex on the digestibility and retention of the nutrients of a hay ration. *J. Agric.* Sci., **43:** 281.
HEMSLEY, J. A. 1964. The utilization of urea-supplemented roughages. *Proc. Aust. Soc. Animal Prod.* **5:** 321.
HEMSLEY, J. A. 1966. Dietary cellulose content and urea utilization. *Proc. Aust. Soc. Animal Prod.* **6:** 384.
HENDERICKS, H. 1961. The incorporation of sulfate in the ruminal proteins. *Archives Internationales de Physiologie et de Biochimie.* **69:** 449.
HOGAN, J. P. 1961. The absorption of ammonia through the rumen of sheep. *Aust. J. Biological Sci.*, **14:** 488.
HOGUE, D. E. 1968. The nutritional requirements of lactating ewes. Proc. Symposium on Sheep Nutrition and Feeding. Iowa State University.
HOLTER, J. B., N. F. COLOVOS and W. E. URBAN, Jr. 1968. Urea for lactating dairy cattle. IV. Effect of urea versus no urea in the concentrate on production performance in a high producing herd. *J. Dairy Sci.*, **51:** 1403.
HOUPT, T. R. 1959. Utilization of bllod urea in ruminants. *Am. J. Physiol.* **197:** 115.
HOUPT, T. R. and K. A. HOUTPT. 1968. Transfer of urea nitrogen across the rumen wall. *Am. J. Physiol.* **214:** 1296.
HUBER, J. T., R. A. SANDY. 1965. Response of dairy cows fed unlimited corn silage to three levels of urea and grain. *J. Animal Sci.*, **24:** *887.*
HUHTANEN, C. N. and L. D. GALL. 1955. Manometric estimation of rumen urease. *J. Bacteriology.* **69:** 102.
HUME, I. D., R. J. MOIR and M. SOMERS. 1970. Synthesis of microbial protein in the rumen. I. Influence of the level of nitrogen intake. *Aust. J. Agric. Research.* **21:** *283.*
HUME, I. D. 1970. Synthesis of microbial protein in the rumen. II. A response to higher volatile fatty acids. *ibid.*: 297.
——— III. The effect of dietary protein. *ibid.*: 305.
HUME, I. D. and P. R. BIRD. 1970. Synthesis of microbial protein in the rumen. IV. The influence of the level and form of dietary sulfur. *ibid.*: 315.
HUNGATE, R. E. 1966. *The Rumen and its Microbes.* Academic Press. New York.
HUNT, C. H., O. G. BENTLEY, T. V. HERSHBERGER AND J. H. CLINE. 1954. The effect of carbohydrates and sulfur on B-vitamins synthesis, cellulose digestion and urea utilization by rumen microorganisms «in vitro.» *J. Animal Sci.*, **13:** 570.
JOHNS, A. T. 1955. Pasture quality and ruminant digestion. II. Levels of volatile fatty acids and ammonia in the rumen of sheep on a high production pasture. *New Zealand J. Sci. Technol.* A. **37:** 323.
JOHNSON, B. C., T. S. HAMILTON, H. H. MITCHELL and W. B. ROBINSON. 1942. The relative efficiency of urea as a protein substitute in the ration of ruminants. *J. Animal Sci.*, **1:** 236.
JOHNSON, R. R. and K. E. MCCLURE. 1964. «In vitro» and «in vivo» comparisons on the utilization of urea, biuret and diammonium phosphate by sheep. *J. Animal Sci.*, **23:** 208.
JORDAN, R. M. 1952. Effect of urea on pregnant ewes. *J. Animal Sci.*, **11:** 768.
KARR, M. R., U. S. GARRIGUS, E. E. HATFIELD and H. W. NORTON. 1965. Factors affecting the utilization of nitrogen from different sources by lambs. *J. Animal Sci.*, **24:** 459.
KURELEC, V. 1959. Urea as a source of protein for ewes. (Translated title) in *Nutrition Abstracts and Review.* 1961. Vol. **31** Abstr. 1538.
LASSITER, C. A., R. M. GRIMES, C. W. DUNCAN and C. F. HUFFMAN. 1958. High level urea feeding to dairy cattle. 3. Effect on performance and metabolism of lactating dairy cows. *Quart. Bull. Michigan Agric. Exp. Sta.* **41:** 326.
LAWLOR, M. J., W. H. SMITH and W. M. BEESON. 1965. Iron requirements of the growing lamb. *J. Animal Sci.*, **24:** 742.

LEWIS, D. 1954. The reduction of sulphate in the rumen of the sheep. *The Biochemistry J.*, **56:** *391.*
LEWIS, D. 1955. Amino acids metabolism in the rumen of the sheep. *Brit. J. Nutrition.* **9:** *215.*
LEWIS, D. 1957. Blood urea concentration in relation to protein utilization in the ruminant. *J. Agric. Sci.*, **57:** 438.
LEWIS, D., K. J. HILL and E. F. ANNISON. 1957. Studies on the portal blood of sheep. I. Absorption of ammonia from the rumen of the sheep. *The. Biochemical J.*, **66:** 587.
LEWIS, D. and I. W. MCDONALD. 1958. The inter-relationships of individual proteins and carbohydrates during fermentation in the rumen of the sheep. I. The fermentation of casein in the presence of starch or other carbohydrate materials. *J. Agric. Sci.*, **51:** 108.
LEWIS, D. 1960. Ammonia toxicity in the ruminant. *J. Agric. Sci.*, **55:** 111.
LEWIS, D. and R. S. EMERY. 1962. Intermediateproducts in the catabolism of amino acids by rumen microorganisms. *J. Dairy Sci.*, **45:** 1363.
LITTLE, C. O., G. E. MITCHELL Jr. and G. D. POTTER. 1968. Nitrogen in the abomasum of wethers fed different protein sources. *J. Animal Sci.*, **27:** 1722.
LOFGREEN, G. P., J. K. LOOSLI and L. N. MAYNARD. 1947. The influence of protein source upon the nitrogen retention by sheep. *J. Animal Sci.*, **6:** 43.
LOFGREEN, G. P., W. C. WEIR and J. F. WILSON. 1953. Gains in weight, nitrogen retention and wool growth of lambs fed a ration containing urea supplemented with sodium sulfate. *J. Animal Sci.*, **12:** 347.
LOOSLI, J. K. and L. E. HARRIS. 1945. Methionine increases the value of urea for lambs. *J. Animal Sci.*, **4:** 435.
LOOSLI, J. K. 1952. Meeting the sulfur requirements of ruminants. *Feed. Age*, **2:** 44.
LOOSLI, J. K. and T. C. CAMBELL. 1961. Nitrogen utilization dan requirements in ruminants. *Proc. Cornell Nutrition Conference.*
LOOSLI, J. K. and I. W. MCDONALD. 1968. Non protein nitrogen in the nutrition of ruminants. *FAO Agricultural Studies* No. 75. Rome.
MAYNARD L. A. and J. K. LOOSLI. 1969. *Animal Nutrition.* 6t edt. McGraw-Hill Book Company, New York.
MCDONALD, I. W. 1948. The absorption of ammonia from the rumen of the sheep. *Biochemical J.*, **42:** 584.
MCDONALD, I. W. 1952. The role of ammonia in ruminal digestion of protein. *The Biochemical J.*, **51:** 86.
MCDONALD, I. W. 1968. Nutritional aspects of protein metabolism in ruminants. *Aust. Veterinary J.*, **44:** 145.
MCINTYRE, K. H. and V. J. WILLIAMS. 1970. The effects of intravenous urea infusions on nitrogen metabolism in sheep. *Aust. J. Agric. Research.* **21:** 95.
MCLAREN, G. A., G. C. ANDERSON, J. A. WELCH, C. D. CAMPBELL and G. S. SMITH. 1959. Diethylstilbestrol and length of preliminary period in the utilization of crude biuret and urea by lambs. *J. Animal Sci.*, **18:** 1319.
MCLAREN, G. A., G. C. ANDERSON, J. A. WELCH, C. D. Campbell and G. S. Smith. 1960. Diethylstilbestrol and length of preliminary period in the utilization of crude biuret and urea by lambs. II. Various aspects of nitrogen metabolism. *J. Animal Sci.*, **19:** 44.
MCLAREN, G. C., G. C. ANDERSON, J. B. PETERS, R. P. DOWDY and K. M. BARTH. 1962. Readily fermentable carbohydrates and the adaptation response. *J. Animal Sci.*, **21:** 1005 (Abstr.).
MCLAREN, G. A., G. C. ANDERSON, L. I. TSAI and K. M. BARTH. 1965. Level of readily fermentable carbohydrate and adaptation of lambs to all-urea supplemented diets. *J. Nutrition.* **87:** 331.
MCNAUGHT, M. L., E. C. OWEN and J. A. B. SMITH. 1950. The utilization of non-protein nitrogen in the bovine rumen. 6. The effect of metals on the activity of the rumen bacteria. *The Biochemical J.*, **46:** 36.
MILLS, R. C., A. N. BOOTH, G. BOHSTEDT and E. B. HART. 1942. The utilization of urea by ruminants as influenced by the presence of starch in the ration. *J. Dairy Sci.*, **25:** 925.
MILLS, R. C., C. C. LARDINOIS, I. W. RUPEL and E. B. HART. 1944. Utilization of urea and growth of heifer calves with corn molasses or cane molasses as the only readily available carbohydrate in the ration. *J. Dairy Sci.*, **27:** 571.
NELSON, A. B., M. G. GREELEY and W. D. CAMPBELL. 1957. Protein supplements for wintering beef cattle. *J. Animal Sci.*, **16:** 1085 (Abstr.).
NOBLE, R. L., L. S. POPE W. D. GALLUP. 1955. Urea and methionine in fattening rations for lambs. *J. Animal Sci.*, **14:** 132.
N. R. C. 1964. *Nutrient requirements of domestic animals. No. 5 Nutrient requirements of sheep.* National Research Council, Washington, D. C.
OLTJEN, R. R., E. F. SMITH, B. A. KOCH and F. H. BAKER. 1959. The value of supplemental trace minerals in cattle fattening rations. *J. Animal Sci.*, **18:** 1196.

OLTJEN, R. R., R. J. SIRNY and A. D. TILLMAN. 1962. Effect of three levels of minerals and three levels of cellulose on the performance of sheep fed purified rations. *J. Animal Sci.*, **21:** 302.
OLTJEN, R. R., and R. E. DAVIS. 1963. Zinc, urea and buffers in all concentrate steer rations. *J. Animal Sci.*, **22:** 842 (Abstr.).
OLTJEN, R. R. and P. A. PUTAAM. 1966. Plasma amino acids and nitrogen retention by steers fed purified diets containing urea or isolated soy protein. *J. Nutrition.* **89:** 385.
OLTJEN, R. R. 1969. Effects of feeding ruminants non protein nitrogen as the only nitrogen source. *J. Animal Sci.*, **28:** 673.

PALADINES, O. L. 1963. Energy utilization by sheep as influenced by physical form, composition and level of intake of diet. PhD Thesis. Cornell University.
PALIAN, B., and B. MARKOTIC. 1962. Testing the strengthening of feed ration of milking sheep with non-protein nitrogen of carbamides. *Veterinaria* (Sarajevo). **11:** 244.
PAYNE, E. and J. G. MORRIS. 1969. The effect of protein content of the diet on the rate of urea formation in sheep liver. *Biochemistry J.*, **113:** 659.
PEARSON, R. M. and J. A. B. SMITH. 1943. The utilization of urea in the bovine rumen. 3. The synthesis and breakdown of protein in rumen ingesta. *Biochemical J.*, **37:** 153.
PEIRCE, A. W. 1951. The influence of the amount of starch on the utilization of urea by sheep. *Aust. J. Agric. Research.* **2:** 446.
POPE, L. S., W. D. GALLUP and C. K. WHITEHAIR. 1952. The value of urea in rations for ewes during gestation and lactation. *Okla. Agric. Exp. Sta. M. P.* **27:** 49.
POPE, L. S., F. BAKER, W. D. GALLUP and C. K. WHITEHAIR. 1952. Urea and cottonseed meal as supplements for lamb-fattening rations. *Okla. Agric. Exp. Sta. M. P.* **27:** 53.
POPE, L. S., W. D. GALLUP and D. C. READ. 1952. Urea in rations for pregnant and lactating ewes. *J. Animal Science.* **11:** 773 (Abstr.).
POPE, L. S., W. D. GALLUP and D. C. READ. 1953. Nutritional studies with pregnant and lactating ewes. *Okla. Agric. Exp. Sta. M. P.* **31:** 26.
PRESTON, R. L., D. D. SCHNAKENBERG and W. H. PFANDER. 1965. Protein utilization in ruminants. 1. Blood urea nitrogen as affected by protein intake. *J. Nutrition.* **86:** 281.
PRESTON, R. L. 1966. Protein requirements of growing-finishing cattle and lambs. *J. Nutrition.* **90:** 157.
PRESTON, R. L. 1968. Reduction of plasma urea-N by diethylstilbestrol in ruminants. *Proc. Soc. Biol. Med.*, **129:** 250.
PURSER, D. B. 1970. Nitrogen metabolism in the rumen: microorganisms as a source of protein for the ruminant animal. *J. Animal Sci.*, **30:** 988.

REID, J. T. 1953. Urea as a protein replacement for ruminants: a Review. *J. Dairy Sci.*, **35:** 955.
REPP, W. W., W. H. HALE and W. BURROUGHS. 1955. The value of several non-protein nitrogen compounds as protein substitutes in lamb fattening rations. *J. Animal Sci.*, **14:** 901.
ROJAS, M. A., I. A. DYER and W. A. CASSAT. 1965. Manganese defiiciency in the bovine. *J. Animal Sci.*, **24:** 664.
ROZGONI, I. I. 1960. Assimilation of sulfate sulfur in the rumen of urea-fed cows. Dopovidi Akad. Nauk. Ukr. RSR 1187 (Cited in *Chem. Abstr.* 1961. **56:** 1468b).
RUPEL, I. W., G. BOHSTEDT and E. B. Hart. 1943. The comparative value of urea and linseed meal for milk production. *J. Dairy Sci.*, **26:** 647.

SANFORD, H. and C. SHEARD. 1929. A photoelectric hemoglobinometer. *J. Laboratory and Clinical Medicine.* **14:** 558.
SATTAROV, D. H. I. 1965. Urea is suitable for ewes and lambs. Ovcevodstvo, 9: 24 (Cited in *Nutr. Abstr. and Review*, 1966. **36:** 3445).
SCALES, F. M. and A. P. HARRISON. 1920. Boric acid modification of the kjeldahl method for crop and soil analysis. *J. Ind. Eng. Chem.* **12:** 350.
SCHAADT, H. Jr., R. R. JOHNSON and K. E. MCCLURE. 1966. Adaptation to and palatability of urea, biuret and diammonium phosphate as NPN sources for ruminants. *J. Animal Sci.*, **25:** 73.
SIMONNET, H., H. LE BARS and J. MOLLE. 1957. Le cycle de l'uree administree par voie bucale chez les ruminants. *Compt. Rend.* **244:** 943.
SLEN, S. B. and F. WHITING. 1955. Wool and lamb production as affected by the source of protein in the ration of the mature ewe. *J. Animal Sci.*, **14:** 844.
SMITH, G. S., R. S. DUNBAR, G. A. MCLAREN, G. C. ANDERSON and J. A. WELCH. 1960. Measurement of the adaptation response to urea nitrogen utilization in the ruminant. *J. Nutrition.* **71:** 20.
SMITH, R. H. 1969. Reviews of the progress of dairy science. Nitrogen metabolism and the rumen. *J. Dairy Research.*, **36:** 313.

SOMERS, M. 1961. Factors influencing the secretion of nitrogen in shhep saliva. 1. The distribution of nitrogen in the mixed and parotid saliva of sheep. Aust. *J. Exp. Biol. and Med. Sci.*, **39:** 111.
2. The influence of nitrogen intake upon blood urea nitrogen and upon the total nitrogen and urea nitrogen in the parotid saliva of sheep. *Ibid.*: 123.
3. Factors affecting the nitrogen fractions in the parotid saliva of sheep with special reference to the influence of ammonia production in the rumen and fluctuations in level of blood urea. *Ibid.*: 133.
4. The influence of injected urea on the quantitative recovery of urea in the parotid saliva and the urinary excretions of sheep. *Ibid.*: 145.
STARKS, P. B., W. H. HALE, U. S. GARRIGUS and R. M. FORBES. 1953. The utilization of feef nitrogen by lambs as affected by elemental sulfur, sulfate sulfur and methionine. *J. Animal Sci.*, **13:** 249.
STARKS, P. B., W. H. HALE, U. S. GARRIGUS, R. M. FORBES and M. F. JAMES. 1954. Response of lambs fed varied levels of elemental sulfur, sulfate sulfur and methionine. *J. Animal Sci.*, **13:** 249.
STEEL, R. G. D. and J. H. TORRIE. 1960. *Principles and Procedures of Statistics*. McGraw-Hill Book Company, Inc. New York.
SWENSON, M. J. 1970. Physiologic properties, cellular and chemical constituents of blood. in Dukes *Physiology of Domestic Animals*. M. J. Swenson ed. 8th edition. Comstock Publishing Assoc., Ithaca.
TAGARI, H., Y. DRORI, I. ASCARELLI and A. BONDI. 1964. The influence of levels of protein and starch in rations of sheep on the utilization of protein. *Brit. J. Nutrition.* **18:** 333.
TELLE, P. P., R. L. PRESTON, L. D. KINTNER and W. H. PFANDER. 1964. Definition of the ovine potassium requirement. *J. Animal Sci.*, **23:** 59.
THOMAS, 0.0., D. C. CLANTON and F. S. WILLSON. 1953. Efficiency of urea utilization as influenced by mineral constituents in a wintering ration for beef steers. *J. Animal Sci.*, **12:** 933 (Abstr.).
THOMPSON, N. R., G. C. GRAF, J. F. EHEART and C. W. HOLDAWAY. 1952. The utilization of urea by dairy cattle. *J. Dairy Sci.*, **35:** 1010.
THORNTON, R. F. 1970. Urea excretion in ruminants. I. Studies in sheep and cattle offered the same diet. *Aust. J. Agric. Res.*, **21:** 323.
II. Studies in sheep whose rumen contents were replaced with physiological saline. *Ibid.*: 337.
THORNTON, R. F., P. R. BIRD, M. SOMERS and R. J. MOIR. 1970. Urea excretion in ruminants. III. The role of the hind-gut (caecum and colon). *Ibid.*: 345.
THORNTON, R. F. 1970. Factors affecting the urinary excretion of urea nitrogen in cattle. I. Sodium chloride and water loads. II. The plasma urea nitrogen concentration. *Aust. J. Agric. Res.*, **21:** 131.145.
TILLMAN, A. D. and K. S. SIDHY. 1969. Nitrogen metabolism in ruminants: Rate of ruminal ammonia production and nitrogen utilization by ruminants. A review. *J. Animal Sci.*, **28:** 689.
VARADY, J., K. BODA, I. HAVASSY, M. BAJO and J. THOMAS. 1967. The relationship between urea retention to ammonia concentration in the rumen of the sheep after intravenous administration of urea. Physiol. Bohemoslov, 16 : 571 (Cited in *Nutr. Abstr. and Reviews*, 1968. **38:** 7272).
VERCOE, J. E. 1969. The transfer of nitrogen from the blood to the rumen in cattle. *Aust. J. Agric. Res.*, **20:** 191.
VIRTANEN, A. I. 1966. Milk production of cows on protein-free feed. *Science.* **153:** 1603.
VIRTANEN, A. I. 1967. New views in cattle feeding: normal concentrates replaced by urea and hemicellulose syrup prepared from wood. *Agrochimica.* **11:** 289.
VIRTANEN, A. I. 1968. Some central nutritional problems of the present time. *Federation Proc.*, **27:** 1374.
VIRTANEN, A. I. 1969. On nitrogen metabolism in milking cows. *Federation Proc.*, **28:** 232.
VISEK, W. J. 1968. Some aspects of ammonia toxicity in animal cells. *J. Dairy Sci.*, **51:** 286.
WALDO, D. R. 1968. Symposium: nitrogen utilization by the ruminant. Nitrogen metabolism in the ruminant. *J. Dairy Sci.*, **51:** 265.
WALKER, D. J. and C. J. NADER. 1968. Method for measuring microbial growth in rumen content. *Applied Microbiology.* **16:** 1124.
WEGNER, M. I., A. N. BOOTH, G. BOHSTEDT and E. B. HART. 1940. The «in vivo» conversion of inorganic nitrogen to protein by microorganisms from the cow's rumen. *J. Dairy Sci.*, **23:** 1123.
WELCH, J. A., G. C. ANDERSON, G. A. MCLAREN, C. D. CAMPBELL and G. S. SMITH. 1957. Time,

doethylstilbestrol and vitamin B_{12} in the adaptation of lambs to NPN utilization. *J. Animal Sci.*, **16:** 1034.
WHANGER, P. D., and G. MATRONE. 1965. Effect of dietary sulfur upon the fatty acid production in the rumen. *Biochim. Biophys. Acta.* **98:** 454.
WHANGER, P. D. and G. MATRONE. 1966. Eeffect of dietary sulfur upon production and absorption of lactate in sheep. *Biochim Biophys. Acta.* **124:** 273.
WHANGER, P. D. and G. MATRONE. 1967. Metabolism of lactic, succinic and acrylic acids by rumen microorganisms from sheep fed sulfur adequate and sulfur deficient diets. *Biochim. Biophys. Acta.* **136:** 27.
WHANGER, P. D. 1969. Sulfur in ruminant nutrition. Metabolism and importance. *The. sulfur Institute J.*, **4:** 9.
WILLMAN, J. P., F. P. MORRISON and E. W. KLOSTERMAN. 1946. Cornell University Agric. Exp. Sta. *Bulletin 834.*
YAMOOR, M. Y., J. C. MEISKE and R. D. GOODRICH. 1968. Adaptation studies with lambs fed urea or biuret. *J. Animal Sci.*, **27:** 1180 (Abstr.).

BIOGRAPHICAL SKETCH

Francisco Javier Ovejero, was born on May 28, 1942, in Valladolid, Spain. He attended private elementary and high schools in León, graduating in September 1958. In the Fall of that year, he entered the Veterinary College of the University of Oviedo at León, from which he graduated with the D. V. M. degree in September of 1964. The Fall of that year he was granted a Fellowship from the spanish «Comisaria de Protección Escolar» and he worked as a Teaching Assistant in the Department of Animal Nutrition at the mentioned Veterinary College of the University of Oviedo at León, where he graduated with the Doctor of Science Degree in Veterinary Medicine in May 1967.

In the Summer of 1967 he was accepted by the Graduate School of Cornell University, where he was granted a Teaching and Research Assistantship in the Department of Animal Science, which together with a Fulbright Travel Grant enabled him to enroll at Cornell University in the Fall of 1967, and since then he has been working towards the Doctor of Philosophy Degree with a major in Nutrition and Minors in Animal Science and Agronomy.