1. General introduction

Availability of quality forage is a major problem in ruminant production in tropical countries particularly during the dry season. At this season forage is expensive and out of reach to many farmers. As a result, there is over-slaughtering and selling of ruminant livestock when the production cost is elevated by the price of forage. Utilisation of other abundant agricultural by-products is one of the alternatives to substitute expensive forage to keep the ruminant body weight losses at the minimum level.

Some fibrous feed from agricultural by-products such as fresh or treated palm press fibre (PPF), rice straw, and sugarcane-bagasse have been used extensively. Cocoa pod, a by-product of cocoa plantations is a potential source of fibre for ruminants. It is easily available to the farmers. In most of tropical regions where the cocoa trees are cultivated (Figure 1.1), the availability of by-products such as cocoa pods increases with the escalation of world-wide demand of cocoa. Cocoa bean production in 2003 forecasted about 3 million tons (ICCO, 2003) which released roughly 6 million tons of cocoa pod meal (a 1 to 2 cocoa bean to cocoa pod meal ratio (DUKE, 1983)), an equivalent of about 25 million tons of fresh cocoa pods if 20% DM are assumed.

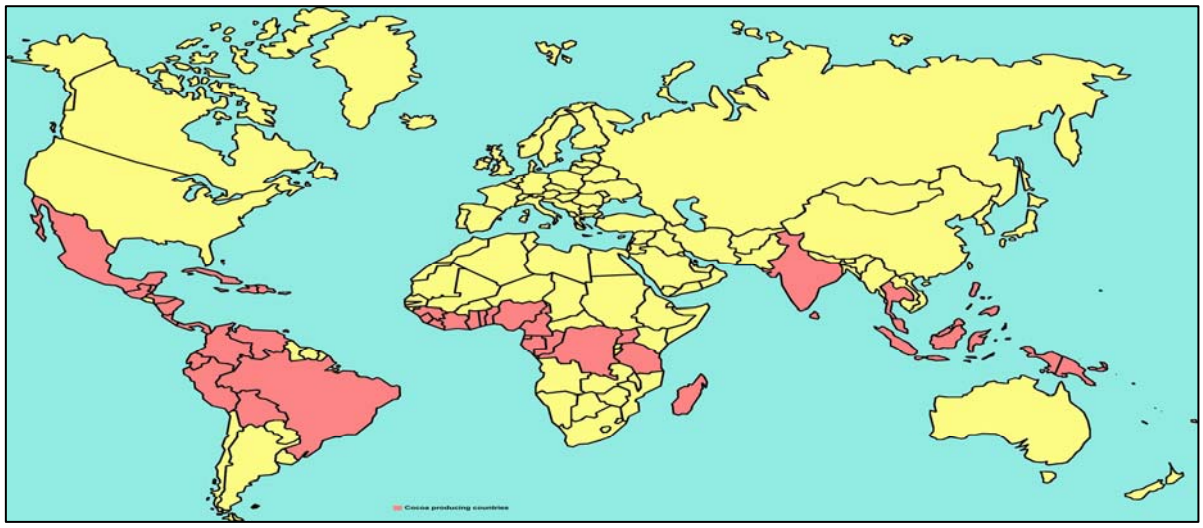

Figure 1.1: Cocoa producing countries (■ producing country)
Source: ICCO (2001)

Cocoa pods are palatable. The low protein content and high cell wall constituents, however, limit the use of cocoa pods in ruminant nutrition (reviewed by REYNOLDS, 1995). As a late-maturing plant component, the pod contains high lignocellulosic and low non-polysaccharide substances. To be used as ruminant feedstuff, cocoa pod needs quality upgrading.

The use of physical, chemical and biological treatments to improve the quality of agricultural by-products has been reviewed by FLACHOWSKY et al. (1999). Alkali agents can cleave lignocelluloses into lignin and cellulose or hemicelluloses. Ammonia and urea, a group of alkali agents, have been reported to be effective in improving fibrous feed quality (SCHIERE & IBRAHIM, 1989; SUNDSTØL et al, 1993; CHENOST & KAYOULI, 1997). In addition to improving digestibility, ammonia treatment may also supply N for microbial growths. The use of ammonia is, however, limited to industrial countries.

Urea as a source of ammonia can be applied on industrial scale as well as at farm level. Compared to other forms of chemical treatment, there are little health risks associated with the use and handling of urea. Dry urea can be easily transported compared to other sources of ammonia. Improvement of fibrous feed quality through urea treatment is due to two processes that take place simultaneously. Firstly, ureolysis splits urea into ammonia. The generated ammonia reacts with H_2O and then acts on the cell walls of the forage (CHENOST, 2001).

Urea treated feed can not be evaluated based on crude protein (CP) analysis only. This is because CP analysis according to the Kjeldahl-method (N x 6.25) is unable to distinguish between true protein and non protein nitrogen and thus to evaluate the contribution of the microbial protein produced in the rumen, which in treated feed diet maybe produced from NPN as the main contribution to the intestinal protein flow (HVELPLUND, 1998). The influence of treatment on digestible crude protein (DCP) is also inadequate for analysis of urea treated feeds because non protein nitrogen (NPN), a high CP fraction in urea treated feed, is not directly related to animal performance (ORSKOV, 1982). Therefore, expressing N-fractions as true protein, non ammonia nitrogen and residual urea is needed.

Urea is a weak alkali. As a result, higher levels of urea are needed to give the same effect on fibre fraction than when using concentrated alkali. Too much urea, on the other hand, can be toxic to ruminants. Urea toxicity and ammonia poisoning occur when blood ammonia levels exceed beyond the buffering capacity of blood. Excessive levels result in a rise in pH and impairment in the capacity of blood to expel carbon dioxide (VAN SOEST, 1982).

Chemical and short in vitro evaluations of urea treated feeds are inadequate to accurately determine long time effects of urea and ammonia in ruminants. Long-term in vitro such as Rusitec or in vivo experiments to study rumen physiology are needed. In Rusitec, the effect of urea can be studied by gradually substituting a conventional ration ingredient with urea treated feed.

Since microbial protein is the major protein contributor for ruminants particularly if urea treated roughage is used in diets, information about the microbial protein synthesis (MPS) and its efficiency (EMPS) are important criteria needed for ration formulation and the estimation of animal performance.

Several methods for assessing MPS have been developed. The majority of the approaches that identify microbial protein in rumen contents (both in vitro and in vivo) and in digesta flowing at the omasum, abomasums or duodenum, have limitations (DEWHURST et al., 2000). Previous work used protein-free diets and this still can provide a useful baseline against which to asses other markers (ARAMBEL et al., 1987). Other early studies tried to distinguish feed and microbial protein on the basis of amino acid profile (ANNISON, 1975; OFFER et al., 1978). Endogenous or exogenous markers to label microbial material including ^{35}S (HUME, 1975; DURAN et al., 1975), ^{32}P (VAN NEVEL et al., 1975), ^{15}N (BRANDT, 1979; ABEL et al., 1990; HRISTOV, 2002), diaminopimelic acid (THOMAS, 1973; MILLER, 1982), RNA (BATES et al., 1985) and purine and pyrimidine bases (SHEM et al., 1999; RODRIGUEZ et al., 2000) have been used. Near infrared reflectance spectroscopy as a quick approach to distinguish the microbial content of duodenal digesta has been used successfully by LEBZIEN & PAUL (1997).

DEWHURST et al. (2000) concluded that each of these markers has its own problems including safety (radioisotopes), cost (^{15}N and amino acid profiles), difficulty of analysis (RNA and DNA) and presence of feeds, thereby lacking specificity to the microbial fraction (DAPA, nucleic acid and their bases).

1.1. Objectives

The general objective of this study was to improve the quality of cocoa pods to allow increased levels of their incorporation into ruminant rations. The first step of the study was directed to find the basic information on changes in urea treated compared to untreated cocoa pod. The in vitro gas test aimed at studying the effect of urea on the activity of rumen

microbes, at finding the optimum level to achieve maximum organic matter digestibility and metabolisable energy contents as well as to find out its toxic level.

The effect of urea treated cocoa pods on rumen microbial metabolism was then investigated by means of the rumen simulation technique Rusitec (CZERKAWSKI & BRECKENRIDGE, 1977). Barley/soybean meal-mixture was gradually substituted for urea treated cocoa pods and nutrient disappearance rates, fermentation patterns as well as microbial growth were measured in order to get more information on the optimal inclusion of urea treated cocoa pod into feed rations for the ruminant animal.

In the last experiment, two methods of determining MPS in cocoa pod-supplied Rusitec were applied and compared; the indirect continuous tracer infusion technique and the direct measurement of isolated microbes. With both methods stable isotope nitrogen (^{15}N) served as a tracer.

2. Cocoa (*Theobroma cacao,* L).

2.1. Classification

According to the PURSEGLOVE (1968), the botanical taxonomy of cocoa is as follows:

Division : Magnoliophyta
Class : Magnoliopsida
Subclass : Dilleniidae
Order : Malvales
Family : Sterculiaceae
Species : *Theobroma cacao*, L

Several subspecies and forms of cocoa have been recognised, from which a great number of cultivars have been developed. Some cultivars are named according to the place where they were found or developed. Others with elongated, ridged and pointed fruits and white cotyledons are classified as **Criollo** typed while those with short, roundish, almost smooth fruits and purplish cotyledons are categorised as **Forastero**, (DUKE, 1983). Morphology and anatomy of cocoa fruit and cocoa flower is shown in figure 2.1.

2.2. History and spreads

Although it is unknown exactly how or when cocoa was discovered in the wild, it is thought that the Aztec and Maya Indians, who called it the "food of the gods", domesticated and cultivated it for centuries before the discovery of the Western Hemisphere (PURSEGLOVE, 1968). It is also believed that the cocoa tree (*Theobroma cacao*) originated from the headwaters of the Amazon Basin and in early times was spread throughout the central part of Amazonia, Guinea, westward and northward to the south of Mexico (WOOD & LASS, 1985). Cocoa then has been extensively cultivated in the Old World since the Spanish conquest (COLUMBIA ENCYCLOPAEDIA, 2003).

The first European to discover cocoa was Columbus although the Spaniard Hernand Cortes was the first person to bring the cocoa seed back to Europe in 1519. In Europe, it was considered as a luxury drink by the West Europe counts (PURSEGLOVE, 1968). It was not until 1657 however, that cocoa was introduced to England, having already been used in Italy, Austria and France (ENCARTA, 1996).

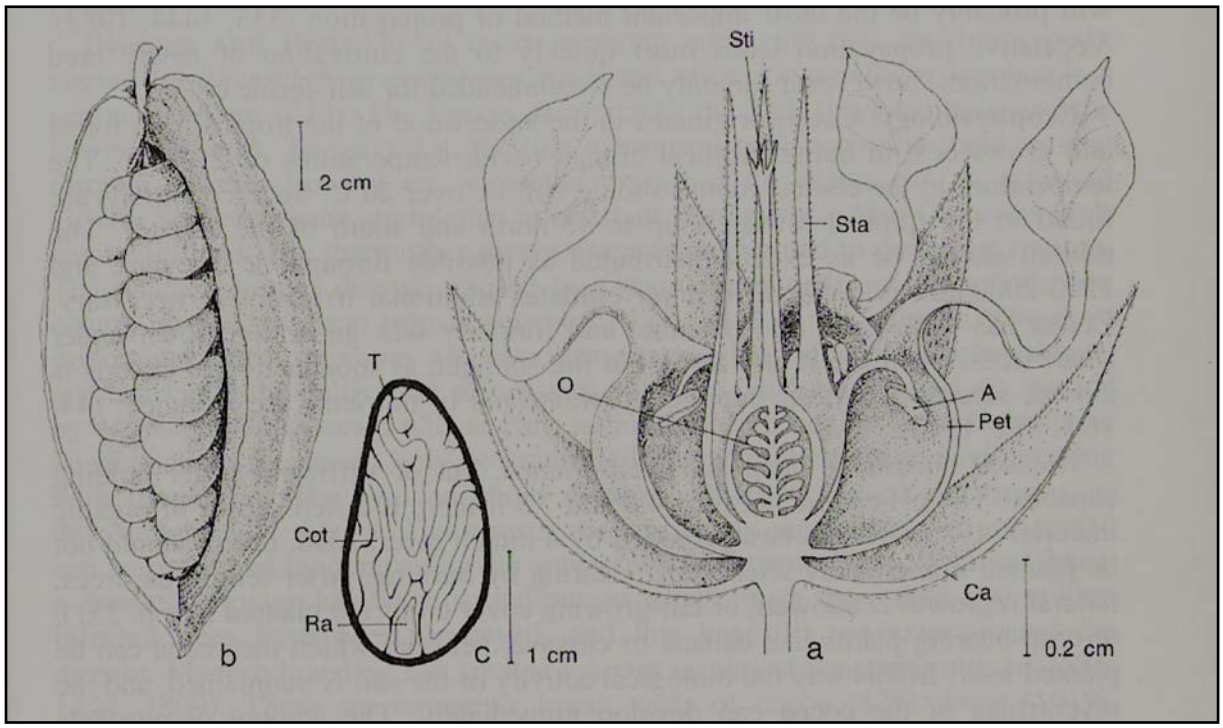

Figure 2.1: Morphology and anatomy of cocoa fruit and cocoa flower *Theobroma cacao*
(a) flower in longitudinal section, (b) opened fruit, (c) seed in longitudinal section,
A = anther, Pet = petal, Cot = cotyledon, O = ovary, Ca = calyx, Sti = stigma,
Ra = radicle, T = staminode; Source: REHM & ESPIG (1991)

In the eighteenth century chocolate started to become available to more classes of society with the establishment of chocolate houses in London, out doing the popularity of the previous centuries of coffee houses. The eighteenth century also saw the English mixing brick dust with their chocolate to thicken it, a habit that was stopped in 1727 when Nicholas Sanders mixed chocolate with milk creating the first hot chocolate. This hot chocolate was promoted by physicians as healthy for both children and adults (YOUNG, 1994).

The cocoa bean was originally roasted, ground and then mixed with maize and annatto, chilly or some other spice, this mixture made a thick drink and was known as chocolate. In this form chocolate became the first non-alcoholic stimulant drink on the European continent (YOUNG, 1994). The Spaniards also realised that the cocoa bean could be made into a delicious drink when mixed with sugar (WOOD & LASS, 1975).

TARLA (1996) summarised the chronology of the cocoa distribution to Europe as listed below:

Found	: Central America
1502, Christopher Columbus	: First European to see cocoa beans but did not show any interest
1519, Hernand Cortes	: First introduced the chocolate drink to the Spanish court.
< 1657, Antonio Carletti	: Italian, spread the chocolate in Italy
1828	: Coenraad van Houten, press 2/3 cocoa butter for better taste
1847	: Fry & Sons, England, introduced eating chocolate
1875	: Daniel Peter, Switzerland, added condensed milk to chocolate
A few years later	: Rodolphe Lindt, invented a way of refining chocolate, it was known as couching process

There have been many and varied uses of the cocoa bean since its discovery. In the early sixteenth century the beans were used for making drinks, currency and payment of tribute to Aztec overlords, and in various rituals and medicinal purposes (WOOD &LASS, 1985).

Chocolate was considered to be a cure for many illnesses and was used for provoking passion. It was, however, expensive for people belonging to the lower class society (Young, 1994).

To date the tree is also found in other tropical areas of the world, such as West Africa, notably Nigeria, Ghana and the Ivory Coast, which produce 2 million tons representing two thirds of the total world crop (ICCO, 2003).