Evaluation of sun-dried *Opuntia ficus-indica* var. Algerian cladodes in sheep diets

by

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Department of Animal, Wildlife and Grassland Sciences,

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Supervisor: Prof. H.O. de Waal (University of the Free State)

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Declaration

I declare that this thesis submitted by me to the University of the Free State for the degree **MAGISTER SCIENTIAE AGRICULTURAE (M.Sc. Agric.)** Animal Science is my own independent work and has not previously been submitted by me for a degree at any other university/faculty. I furthermore cede copyright of the thesis in favour of the University of the Free State.

Desirée Carla Zeeman
Bloemfontein
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Abstract

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The effect of incremental inclusion levels (0, 12, 24 and 36%) of dried and coarsely ground *Opuntia* cladode material in balanced diets to substitute some of the lucerne was investigated with regard to the digestibility of the diets, as well as the effects of the *Opuntia* cladodes on rumen variables (pH, ammonia levels and volatile fatty acid concentration).

The digestibility trial was conducted over a period of 19 days with 24 young Dorper wethers; divided in four treatment groups (T0, T12, T24 and T36) each receiving a different inclusion level of *Opuntia* cladodes. Feed grade urea was included in diets containing *Opuntia* cladodes to compensate for the lower crude protein (CP) content of the *Opuntia* cladodes. Water consumption, food intake and digestibility of the diets were measured.

The inclusion of incremental levels of *Opuntia* cladodes caused a decrease in organic matter (OM), acid-detergent fibre (ADF), neutral-detergent fibre (NDF) and gross energy (GE), while the ether extract (EE) content increased. The water intake of the wethers increased significantly ($P<0.05$) with the increased *Opuntia* cladode content of the diets while urine excretion remained the same. The level of food intake as well as the faeces dry matter (DM) excreted remained the same for all diets but the DM content of the faeces decreased with the higher *Opuntia* cladode inclusion levels. This is ascribed to the mucilage content of the *Opuntia* cladodes. The incremental inclusion levels of *Opuntia* cladodes caused an increase

in apparent DM digestibility coefficient (from 0.673 for diet T0 to 0.716 for diet T36) suggesting that the *Opuntia* cladodes are highly digestible. Due to the low OM content of the *Opuntia* cladodes there was a decrease in the digestible energy (DE) content from diet T0 (17.253 MJ/kg) to diet T36 (12.689 MJ/kg). In spite of these results there appeared to be no discernable difference in overall performance of the sheep on the various diets.

The rumen fermentation was studied with 4 rumen cannulated Dorper wethers in a trial with a crossover design during four successive 14-day periods. The incremental inclusion levels of *Opuntia* cladodes in the diets had no significant ($P<0.05$) influence on the rumen ammonia (NH_3) concentration. The rumen NH_3 concentration was consistently between 9.4 mg NH_3/100 ml rumen fluid and 58.5 mg NH_3/100 ml rumen fluid, with a peak at 2 hours post-feeding. The inclusion of *Opuntia* cladodes in the diets also had no effect on the rumen pH. The rumen pH consistently ranged between 6.3 and 7.2. The inclusion level of *Opuntia* cladodes also had no significant ($P<0.05$) effect on the rumen volatile fatty acids (VFA) concentration or the proportions of the acetate, propionate or butyrate in the total VFA pool in the rumen. There was also now significant ($P<0.05$) difference in the *in sacco* DM disappearance in the rumen, again suggesting no effect on the microbial activity in the rumen.

The use of dried and coarsely ground *Opuntia* cladodes in balanced diets for sheep as partial substitution for coarsely ground lucerne hay to an inclusion level of 36% in the diet is, therefore, a viable option and can decrease the cost of sheep diets. In practical terms the greatest challenge to overcome will be the successful drying of large quantities of *Opuntia* cladodes, thus enabling farmers to transport it over longer distances from the production areas to where it can be used as livestock feed. This study offer some suggestions about cutting and drying of the *Opuntia* cladodes but further research is required to determine what possible influence the processing and drying of the cladodes in the sun may possibly has on its nutritional value.

More research is required on the role and especially the effects of mucilage on the digestive processes in ruminant feeds.

Opsomming

Evaluation of sun-dried *Opuntia ficus-indica* var. Algerian cladodes in sheep diets

deur

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Die invloed van inkrementele insluitingspeile (0, 12, 24 en 36%) gedroogde en grof gemaalde *Opuntia* kladode materiaal in gebalanseerde diëte ter gedeeltelike vervanging van lusern is ondersoek ten opsigte van die verteerbaarheid van die vier diëte, asook die invloed van die *Opuntia* kladodes op rumen veranderlikes (pH, ammoniak- en vlugtige vetsuurkonsentrasies).

'n Verteringsproef is oor 'n periode van 19 dae met 24 jong Dorperhamels uitgevoer wat in vier behandelingsgroepe (T0, T12, T24 en T36) verdeel is en diëte met verskillende insluitingspeile van *Opuntia* kladodes ontvang het. Voergraad ureum is in die diëte wat *Opuntia* kladodes bevat het ingesluit om te kompenseer vir die laer ruproteïeninhoud (RP) van die *Opuntia* kladodes. Die water- en voerinname deur die hamels en die verteerbaarheid van die vier diëte is bepaal.

Die insluiting van inkrementele peile van *Opuntia* kladodes het 'n afname in organiese materiaal (OM), suurbestande vesel (ADF), neutraalbestande vesel (NDF) en bruto energie (BE) tot gevolg gehad, terwyl die eterekstrakinhoud verhoog het. Die waterinname van die hamels het betekenisvol ($P<0.05$) gestyg met toenemende *Opuntia* kladode insluiting in die diëte, terwyl urine uitskeiding onveranderd gebly het. Die voerinname sowel as die droë materiaal (DM) van die misuitskeiding het dieselfde gebly vir die diëte, maar die DM-inhoud van die mis was laer met toenemende *Opuntia* kladode insluitingspeile. Dit word toegeskryf

aan die hoë slymgominhoud ("mucilage") van die *Opuntia* kladodes. Die inkrementele insluitingspeile van *Opuntia* kladodes het 'n toename in die skynbare DM verteerbaarheid koëffisient (vanaf 0.673 vir dieet T0 tot 0.716 vir dieet T36) tot gevolg gehad; dit dui daarop dat *Opuntia* kladodes hoogs verteerbaar is. As gevolg van die laer OM-inhoud van die *Opuntia* kladodes was daar 'n afname in die verteerbare energie (VE) vanaf dieet T0 (17.253 MJ/kg) na dieet T36 (12.689 MJ/kg). Ten spyte van die resultate was daar geen merkbare verskil in diereprestasie van die hamels op die verskillende diëte nie.

Die rumenfermentasie is bestudeer met vier rumengefistuleerde Dorperhamels gedurende vier opeenvolgende periodes van 14 dae in 'n proef met 'n omslagontwerp. Die inkrementele insluitingspeile van *Opuntia* kladodes in die diëte het nie 'n betekenisvolle ($P<0.05$) invloed op die rumen ammoniak (NH_3) konsentrasies gehad nie. Die rumen NH_3 konsentrasies was deurgaans tussen 9.4 mg NH_3/100 ml rumenvloeistof en 58.5 mg NH_3/100 ml rumenvloeistof, met 'n piek 2 uur na voeding. Die insluiting van *Opuntia* kladodes het ook geen betekenisvolle ($P<0.05$) invloed op rumen pH gehad nie; die rumen pH was deurgaans tussen 6.3 en 7.2. Die insluitingspeile van *Opuntia* kladodes het geen betekenisvolle ($P<0.05$) invloed op die konsentrasie van die rumen vlugtige vetsure (VVS) of die verhouding van asyn-, propion- of bottersuur in die totale VVS poel in die rumen gehad nie. Daar was ook geen betekenisvolle ($P<0.05$) verskil in *in sacco* DM verdwyning in die rumen nie, wat weer eens daarop dui dat geen invloed op die mikrobiese aktiwiteit in die rumen uitgeoefen is nie.

Die aanwending van gedroogde en grof gemaalde *Opuntia* kladodes in gebalanseerde diëte vir skape ter gedeeltelike vervanging van grof gemaalde lusernhooi tot 'n insluitingspeil van 36% in die dieet is, derhalwe, 'n haalbare opsie en kan die koste van skaapdiëte verminder. In praktiese terme is die belangrikste uitdaging wat oorbrug moet word die suksesvolle droging van groot hoeveelhede *Opuntia* kladodes om boere sodoende in staat te stel om dit oor langer afstande te vervoer na gebiede waar dit as veevoer benut kan word.

Hierdie studie bied voorstelle oor wyses om *Opuntia* kladodes te sny en te droog, maar meer navorsing is nodig om te bepaal wat die moontlike invloed van prosessering en droging van die kladodes in die son op die voedingswaarde mag hê.

Meer navorsing is ook nodig oor die rol en veral die invloed van slymgom op die verteringsprosesse by herkouer diëte.

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1. Introduction

According to Barbera (1995) it took opuntias - one of the most relevant economic plants of the Aztec civilization - centuries to be fully appreciated in the various regions of the world. It is highly sought after as human food, namely as "nopalitos" and various forms of fresh and processed cactus pear fruits, but is also an important source of animal feed (De Kock & Aucamp, 1970; Barbera, 1995; Felker, 1995; Scheinvar, 1995; Pretorius *et al.*, 1997; Ben Salem *et al.*, 2002a; Tegegne, 2002b; Batista *et al.*, 2003).

The ecological success of opuntias and other cacti is partly a reflection of their daily pattern of carbon dioxide (CO_2) uptake and water loss, both of which occur primarily at night; most plants open their stomates, and hence begin taking up CO_2 from the atmosphere, at dawn (Nobel, 1995). The CO_2 taken up is incorporated into various products of photosynthesis, a process that takes place only in light. The opening of stomates also leads to an inevitable loss of water from inside leaves and photosynthetic stems. The opening of the stomates during daytime leads to a much greater water loss than for the same stomatal opening at night, when temperatures are lower and humidity is higher. The opening of the water-conserving stomatal at night means that CO_2 uptake occurs in the dark; this gas exchange pattern is known as crassulacean acid metabolism (CAM) in plants (Nobel, 1995).

Opuntias are particularly attractive as an animal feed because of its high efficiency in converting water to dry matter (DM) and thus digestible energy (Nobel, 1995). Cacti are not just useful because it can withstand drought, but because its conversion efficiency is greater than C_3 grasses and C_4 broadleaves (Felker, 1995). The CAM plants, such as opuntias, represent about 6 to 7% of the nearly 300 000 plant species; most plant species (92 to 93%) are C_3 (whose first photosynthetic product is a 3-carbon organic compound). Only about 1% of the plant species are C_4 plants (whose first photosynthetic product is a 4-carbon organic compound), but such species are quite important ecologically and agronomically and include sugar cane (*Saccharum officinarum*), sorghum (*Sorghum bicolor*), corn or maize (*Zea mays*) and many wild tropical grasses. In comparison with these C_4 crops, as well as with C_3 crops such as alfalfa (*Medicago sativa*), rice (*Oryza sativa*) and wheat (*Triticum vulgare*), CAM plants are generally, and correctly, viewed as very slow growers (Nobel, 1995).

According to Nobel (1995) this low productivity, however, is not an inherent characteristic of the CAM pathway; it does not apply to the CAM species *Opuntia ficus-indica*, which is cultivated in about 30 countries for its fruits, young cladodes (used as a vegetable) and mature cladodes (used for forage and fodder). Even though water conservation is of critical importance for opuntias, other environmental variables such as temperature, light, nutrients and soil salinity, also affect their daily net CO_2 uptake, productivity, reproduction and survival (Nobel, 1995).

The presence of opuntias was first reported in 1772 in South Africa (Barbera, 1995). A distinction is made in South Africa between the green leaf spineless cactus pear (*Opuntia ficus-indica*) varieties and the blue-leafed spineless cactus pear (*Opuntia robusta*) varieties (Felker, 1995). In 1914 the Research Institute at Grootfontein, Middelburg (currently in the Eastern Cape Province of South Africa) introduced 22 spineless cactus pear varieties for use as a livestock fodder crop, following Burbank's work in California (De Kock, 1980; Barbera, 1995; Felker, 1995). Often *Opuntia* species are regarded as weeds, that is, those that were introduced for whatever reason into a country outside their native habitats and became naturalized (Brutsch & Zimmermann, 1995). There is some evidence to suggest that originally (at least 250 years ago) only spineless varieties of *Opuntia ficus-indica* were introduced into South Africa and that these have reverted back to the spiny form over a period of nearly 200 years; the spiny forms are considerably more aggressive than the spineless forms and are therefore better adapted to spread (Brutsch & Zimmermann, 1995). After cactus pears infested about 900 000 ha mainly in the Eastern Cape it was controlled to a large extent during the 20th century with biological control and an act that applied specifically to the spiny form, prohibiting the uncontrolled diffusion of the plants (Barbera, 1995; Brutsch & Zimmermann, 1995).

The cladodes of spiny and spineless cactus pears (*Opuntia* species) are used as feed for livestock during the frequent periods of food shortages or droughts in many arid and semi-arid regions (De Kock & Aucamp, 1970; Felker, 1995; Pretorius *et al.*, 1997; Ben Salem *et al.*, 2002a; Tegegne, 2002b; Batista *et al.*, 2003). In South Africa it has been used by livestock farmers as drought fodder since the 18th century when first introduced to the country (Van Sittert, 2002). Spineless cactus pears are valued by many farmers because of their drought resistance, high biomass yield, palatability and adaptability to a range of soils and climatic regions (Zeeman & Terblanche, 1979; Ben Salem *et al.*, 1996; Batista *et al.*, 2003). Many farmers have spineless cactus pear plantations or orchards on their farms even if it is only a small orchard.

While spineless cactus pear plantations require protection from livestock, the spiny *Opuntia* types, on the other hand, do not require fencing. However, the spines of the spiny *Opuntia* types must be removed or singed off with a flame thrower (the so-called pear burner in Texas) before being fed to cattle or sheep (Felker, 1995). In countries such as for example Texas and northern Mexico, spineless cactus pear plantations must be extremely well protected (2.4 m high netwire fence, with 5 cm mesh at the bottom) against herbivory from rabbits, rats and wildlife, especially deer and peccaries (Felker, 1995).

Production of spineless cactus pear (*Opuntia ficus-indica*) in South Africa for fruit production aimed at export markets in among others countries in Europe, has recently increased considerably (Claassens & Wessels, 1997). During 2003 more than 465 000 kg of fresh fruits were exported by sea and air from South Africa (Anonymous, 2003). The production of spineless cactus pear fruits creates the prospect of utilizing the large quantities of plant material that is yielded annually as pruned waste as a feed source for livestock.

Spineless cactus pears have their limitations and cannot sustain animals indefinitely as the sole feed source (Myburg, 1961). It is not a balanced diet and should be seen as a relatively good and cheap energy source only (Steenkamp, 1973; Nefzaoui & Ben Salem, 2000). Therefore, spineless cactus pear cladodes can play an important role in the supplementary feeding of ruminants during droughts. During these recurring droughts in South Africa the natural pastures or veld is dry and drinking water is limited, thus, spineless cactus pears can provide some of the necessary water required by animals. Trials showed that sheep could be maintained on spineless cactus pear cladodes for more than 300 days without receiving additional water (De Kock, 1965).

The spineless cactus pear is regarded a drought resistant crop that can be considered favourably as feed source since it is easy to maintain, it yield large quantities of edible material for livestock and it also produces fruits that generate additional income. In general, opuntias are considered to be high in water content (about 850 g water/kg fresh material), high in *in vitro* digestibility (about 750 g/kg DM), but low in protein content varying from about 50 to 120 g protein/kg DM (Felker, 1995). Furthermore, while there are substantial data on the protein and mineral contents of opuntias being used to produce fruit, there are surprisingly little digestibility or metabolizable energy data available to assist in formulating animal diets (Felker, 1995). Most of the nutrient deficiencies or shortcomings of opuntias can be rectified easily by appropriate supplementation. However, a serious disadvantage of opuntias is the enormous water content of the cladodes (about 850 g water/kg fresh material) that makes it prohibitively expensive to transport it over long distances. Cacti contain mucilage which is commonly described as water-soluble pectin-like polysaccharide (Cárdenas *et al.*, 1997). The precise function of the mucilage in cactus pears is not known, however, it is generally believed to help retain water in the cactus (Sudzuki Hills, 1995). However, the ability of cacti to retain water under unfavourable climatic conditions of prolonged drought is due in part, at least, to the water-binding capacity of mucilage (Mindt *et al.*, 1975). Therefore, a further advantage is that when spineless cactus pear cladodes are fed to animals they do not need much, if any, additional water.

A distinct concern when feeding spineless cactus pear cladodes to ruminants is the laxative effect (Ben Salem *et al.*, 2002a). Steenkamp (1973) stated that the relatively high potassium (K), magnesium (Mg) and calcium (Ca) contents may cause the laxative action that occurs when animals are fed spineless cactus pear cladodes. However, Nefzaoui and Ben Salem (2000) suggested that the high oxalate content may explain the laxative effect of spineless cactus pear cladodes. This diarrhoea does not seem to have a negative effect on animals except that it decreases the digestibility of the diet slightly and require precautionary measures to guard against increased blowfly attacks. The high water content also inhibits the intake of fresh cladodes by sheep (Steenkamp, 1973; Steenkamp & Hayward, 1981).

Walters (1951) studied the effect of season on the nutrient content of spineless cactus pear cladodes and showed that the water content is higher during winter than summer. Consequently the nutrient content of fresh spineless cactus pear cladodes is lower during winter than in summer, provided the increase in water content is not accompanied by any increase in the more digestible nutrient content. Walters (1951) also showed that spineless cactus pear cladodes contained significantly higher quantities of protein, fat, fibre and ash on a DM basis in winter. The nitrogen free extract (NFE) content of the spineless cactus pear, on the other hand, is lower during winter. On a wet basis, however, the nutrient content in summer exceeded that in winter (Walters, 1951). Furthermore, Walters (1951) reported that with a higher summer rainfall, the NFE content is higher than for a lower rainfall due to better growth; if a wet summer is followed by a mild winter, it results in a markedly higher protein and a lower NFE content, suggesting less of a restriction in growth. The fibre content of the spineless cactus pear is also higher during dry seasons than wet seasons (Walters, 1951).

Spineless cactus pear cladodes are palatable and it is relished by animals, therefore, the plants often have to be protected to prevent damage from overgrazing or over utilization (Turpin & Gill, 1928; Myburg, 1961; Felker, 1995). Spineless cactus pear plantations can be grazed by ruminants while supplementary feed is provided separately in feed troughs (Terblanche, 1970). This is a simple way to utilize spineless cactus pears (Steenkamp & Hayward, 1981) and requires little labour input; therefore, it is considered a cheap method of utilization (Steenkamp, 1973). However, the necessary precautions should be taken to prevent overgrazing of the cactus pear plants by sheep; especially young plants must be protected (De Kock, 1965; Terblanche, 1970; Steenkamp, 1973; Steenkamp & Hayward, 1981). Overgrazing can destroy the young plants and even older plants can be grazed to the extent that they will yield much less during the following season (Steenkamp, 1973). If spineless cactus pear plants are heavily grazed they should only be utilized every second year (De Kock, 1965).

To protect the plants from overgrazing and obtain satisfactory utilization the spineless cactus pear plantation can be divided into small camps and grazing of a camp by sheep allowed intensively for short periods at a time only (Terblanche, 1970; Steenkamp, 1973). Even with intensive utilization for short periods the plants must still be protected from being damaged. Moreover, plants must not be grazed during their first growing season (Steenkamp, 1973). Grazing or harvesting of cladodes should preferably only commence when plants are three years old (De Kock, 1965). When the plants are grazed the first time, grazing should be restricted to the removal of only the two most recently produced cladodes. In subsequent grazing cycles only the current season's growth should be grazed before the animals are removed (De Kock, 1965).

It is recommended that spineless cactus pear cladodes are fed to ruminants by chopping it into small blocks of about 20 to 30 mm (De Kock, 1965; Steenkamp & Hayward, 1981). Cutting the cladodes into strips of 20 to 30 mm is another cheap option of processing (Steenkamp, 1973). A method that requires little labour and is also quick to apply is the use of a portable shredder in the orchard. The slashed or shredded cladodes can be left between the rows in the orchard where sheep will pick it up. This method, however, causes waste and it is suggested that the slashed or shredded material should rather be fed in troughs (Steenkamp, 1973; Steenkamp & Hayward, 1981). Shredding of the spineless cactus pear cladodes will lead to a higher intake and supplementary feed can then also be provided in separate feed troughs (Terblanche, 1970).

Sheep in a moderate to a good body condition can be fed shredded spineless cactus pear cladodes as the sole feed during a relatively extended drought period. However, the spineless cactus pear cladodes will only supply about 64% of the daily energy requirements of sheep. Even if the shredded cladodes are left to wilt and loose some water before being fed to sheep, it will only supply about 70% of the daily energy requirements of sheep (Steenkamp & Hayward, 1981).

Spineless cactus pear cladodes can also be used as a supplement on dry veld for ruminants, especially where shrubs and bush predominate. The feeding of shredded spineless cactus pear cladodes on veld and supplemented with a salt lick will constitute a relatively good diet (Steenkamp & Hayward, 1981). If a relatively large quantity of dry material is still available on Karoo veld and it is supplemented with spineless cactus pear cladodes, no additional feed is required (Steenkamp, 1973).

When fed in the dried and ground form, spineless cactus pear cladodes still meet only about 85% of the daily requirements of sheep (Steenkamp & Hayward, 1981), suggesting that animals will loose body weight at a slower rate when water is first removed from the plant material by drying before it is fed. Chopped spineless cactus pear cladodes can be dried on clean, hard surfaces; a cement surface is probably the ideal (Terblanche *et al.*, 1972; Steenkamp, 1973; Steenkamp & Hayward, 1981). The chopped spineless cactus pear cladodes are strewn on the surface and frequently turned over, preferably on a daily basis with a hay or garden fork. The chopped and dried pieces of spineless cactus pear cladodes are not well eaten by sheep (Steenkamp, 1973). If spineless cactus pear cladodes are chopped and dried it is advisable to ground it to pass through a 6 mm sieve. In the ground form it is not only utilised better but it is also stored more easily in bags. This method also facilitates stockpiling of large quantities of feed reserves that can be utilized during drought (Steenkamp, 1973; Steenkamp & Hayward, 1981). Dried and ground spineless cactus pear cladodes can be mixed with about 30% lucerne meal or about 6.5% fishmeal to constitute a good maintenance diet. The results of trials where spineless cactus pear cladodes have been supplemented with non-protein nitrogen (NPN) as a protein supplement were disappointing (Steenkamp & Hayward, 1981).

De Kock (1980) reported that chopped spineless cactus pear cladodes can be mixed with oat straw, low grade lucerne hay or other roughage in a ratio of 84% fresh spineless cactus pear cladodes and 16% roughage. Two kg of molasses meal is then added per 100 kg of the mixture to produce good quality silage. When spineless cactus pear fruits are used with the cladodes to produce silage, no additional molasses is necessary. The spineless cactus pear silage is used in the conventional manner as a ruminant feed.

Adult sheep need about two weeks to adapt to a diet of fresh, chopped spineless cactus pear cladodes. Once adapted, sheep will consume about 2.3 to 6.8 kg chopped fresh material daily and again, the intake by sheep will only satisfy about 80% of their energy requirements and about 36% of the protein and 32% of the P requirements, while the total Ca requirement will be provided (Terblanche, 1970).

The voluntary DM intake by sheep on spineless cactus pears is less than their maintenance requirements and the animals will loose body mass. The voluntary intake of dried and ground spineless cactus pear cladodes, with a much lower water content than other physical forms of spineless cactus pears, was markedly higher and consequently the loss in body mass was also much less (Jacobs, 1977).

Usually ruminants consume more of diets with higher protein contents. Consequently the low protein content will also inhibit the ingestion of spineless cactus pears, resulting in a low intake of energy. Therefore, some form of protein supplementation is necessary for sheep to utilise the spineless cactus pears more efficiently (Steenkamp, 1973). According to Terblanche (1970) lucerne is a good and cheap way to supplement the low protein content of the spineless cactus pear cladodes. The relatively high fibre content of lucerne also reduces the laxative effect in sheep consuming the spineless cactus pear material. Other legumes with relatively high protein content can also be used (Steenkamp, 1973).

Maize and other high energy containing feeds should not be used to supplement spineless cactus pear material for ruminants. High energy feeds cause a decrease in the spineless cactus pear intake because animals tend to concentrate on the more palatable supplement. More of the usually expensive supplement is then needed to ensure sufficient energy intake by the sheep. The choice of feed source used as supplement will be determined by its price.

Turpin and Gill (1928) concluded that spineless cactus pear cladodes are palatable with a relatively high nutritive value when fed to dairy cattle for a short period of 67 days and it did not taint the milk. Cows can, however, not be maintained on a diet containing only spineless cactus pear cladodes due to its low protein and fibre content (Turpin & Gill, 1928; Albuquerque *et al.*, 2002). Additional feeds are therefore required to prevent a loss in milk production. Serious diarrhoea also occurred when cows were fed spineless cactus pear cladodes but with no visible signs of negative effects on the animals except that they appeared to be more susceptible to cold. The diarrhoea stopped as soon as cows were fed a conventional diet without spineless cactus pear cladodes (Turpin & Gill, 1928).

Turpin and Gill (1928) reported that calves born of cows fed large quantities of spineless cactus pear cladodes did not perform differently from calves whose dams were not fed spineless cactus pear cladodes. However, the effect of spineless cactus pear cladodes on the individual cows differed; some cows reacted well to spineless cactus pear cladodes in the diet while one cow showed a large loss in body weight. Other cows did not utilize it effectively and some did not even eat it at all. Overall it was found that spineless cactus pear cladodes could be fed successfully to dairy cows. It increased milk production but the butter fat content decreased. The spineless cactus pear cladodes also gave the butter a darker colour, even though there was no difference in taste. When fed at high levels it decreased the digestibility of the other feeds (Turpin & Gill, 1928).

The spineless cactus pear fruit industry in South Africa has increased considerably in recent years. Large quantities of fruits are exported annually and this means that large quantities of fresh cladodes also become available when the plants are pruned to stimulate fruit production. These pruned fresh cladodes are to a large extent considered waste material. Most farmers who produce spineless cactus pear fruits feed some of this material to their livestock, but since they are not primarily livestock farmers, they do not keep enough livestock to utilize such large volumes of fresh plant material in a short period of time. Spineless cactus pear cladode silage is produced on a limited scale by some farmers (H.J. Fouché, 2004; personal communication). As discussed previously, a distinct disadvantage of spineless cactus pear cladodes is the high water content; therefore, it is not practical to transport bulky plant material to livestock farmers that may have a need for additional feed. The result is that most of the pruned fresh cladodes that could have been utilized more efficiently as livestock feed are simply chopped slightly and left in the orchards to decay.

An important challenge in utilising large volumes of *Opuntia* cladodes as livestock feed is to develop a method to dry the cladodes effectively; enabling the dried material to be transported from the fruit producing areas to other regions where it is needed as livestock feed. An effective and practical method to dry the bulky material will also enable livestock farmers with smaller spineless cactus pear orchards to store the pruned material easily as a feed source for their animals.

This study was designed to evaluate the inclusion of incremental levels of dried and coarsely ground *Opuntia ficus-indica* var. Algerian cladodes in balanced diets for sheep as partial substitution of coarsely ground lucerne hay in the diets. Lucerne is a popular ingredient in ruminant diets but it may be expensive because of high demand, notably during periods of drought. If substantial quantities of dried and coarsely ground *Opuntia ficus-indica* cladodes can be included in diets without detrimental effect on animal production, the substitution of lucerne in these diets with an alternative feed source (that is considered a waste product by some), may turn it into a valuable livestock feed.

2. Experimental procedures

Cladodes of the spineless cactus pear *Opuntia ficus-indica* var. Algerian was used in this study and is referred to in an abbreviated format as *Opuntia* cladodes.

The study consisted of two separate trials that ran consecutively:

- In the first trial, 24 young Dorper wethers were used to evaluate the feed intake and digestibility of four diets with incremental inclusion levels of dried and coarsely ground *Opuntia* cladodes.
- In the second trial, four (4) rumen cannulated young Dorper wethers were used in an evaluation of rumen variables; at the same time the feed intake and digestibility were also determined for the four diets with incremental inclusion levels of dried and coarsely ground *Opuntia* cladodes.

It is important to provide some background on the rationale and design of this study, comprising two consecutive trials. Feed intake and digestibility trials are customary conducted with a small number of animals. Often these studies are conducted with rumen cannulated animals to facilitate additional studies on rumen variables that are running concurrently with the same few animals during a single trial period (De Waal *et al.,* 1989; De Waal, 1995; Ben Salem *et al.,* 1996). This may be convenient when a small number of animals are involved and the daily activities of feeding and watering, collecting feed refusals, faeces and urine as well as the activities pertaining to the rumen studies can be completed routinely in a relatively short period of time. However, when larger numbers of animals are involved the added stress imposed on the animals may be considerable when conducting the same array of activities over a longer period every day, especially since the animals are already confined and subjected to the stressful environment of metabolism crates. Therefore, in an attempt to minimize unnecessary stress on the wethers, the feed intake and digestibility trial with the four diets and 24 Dorper wethers was conducted independent of a second trial where the focus would be on rumen variables. In the second trial, only four (4) rumen cannulated Dorper wethers were used in a crossover design (Williams, 1949) to evaluate the rumen variables and most important, where it was possible to execute the daily activities in a relatively short period of time, thus limiting additional stress as far as possible.

2.1 Feed intake and digestibility trial

2.1.1 Preparation of feeds

The *Opuntia* cladodes used in the first trial of this study were harvested on 12 May 2004 at a farm in the Bloemfontein district, Free State Province, South Africa. The cladodes produced during the preceding growing seasons of 2002/2003 and 2003/2004 were pruned and harvested from 10 trees in an orchard of fruit producing *Opuntia ficus-indica* var. Algerian. The freshly pruned *Opuntia* cladodes were weighed with a Salter spring balance, yielding 1 159 kg fresh plant material. The *Opuntia* cladodes were packed into empty fertilizer bags and within a few hours transported to the campus of the University of the Free State for further processing.

Over the next two weeks the *Opuntia* cladodes were cut lengthways by hand into strips of approximately 20-25 mm using a sharp, long bladed butchers' knife. The *Opuntia* cladode strips were packed in a single layer but with some space between strips, on wire racks and dried in the sun. This procedure facilitated air movement around the *Opuntia* cladode strips to promote faster drying over a period of about one week. After a week of drying in the early winter sun of the Free State (second half of May 2004), the *Opuntia* cladode strips that were still not very dry were curled up and the cut wounds covered with white callous material, which reduced the effectiveness of the drying process. The partially dried *Opuntia* cladode strips were then ground to pass through a 20 mm sieve in a small hammer mill. It was deliberately decided in this study to use a sieve with a fairly large aperture size of 20 mm and not a sieve with a much smaller aperture size of 6 mm as suggested by Nefzaoui and Ben Salem (2000).

According to Terblanche *et al.* (1971), chopped spineless cactus pear cladodes dried relatively quickly in the sun, reducing the water content to 121 g water per kg chopped cladodes. However, in this trial the *Opuntia* cladode strips were not completely dry even after a week, therefore, the freshly ground material was spread out again on a dry, clean cement floor in the sun for further drying. Plastic sheets proved to be unsuitable for drying the material because the plastic did not absorb the moisture that evaporated from the coarsely ground *Opuntia* cladodes; the moisture tends to condensate on the plastic, causing the material at the bottom to remain wet or even become wetter while the material at the top dries slightly. Therefore, the coarsely ground and partially dried *Opuntia* cladode strips were further dried on a dry, clean cement surface while it was turned over frequently to prevent it from moulding.

After the first grinding process through the hammer mill some larger pieces of *Opuntia* cladode strips passed almost unaffected through the 20 mm sieve because they were still relatively moist and thus flexible when ground the first time. Therefore, it was necessary to ground the coarsely ground and by now much drier plant material a second time to produce more homogenous material.

A major goal of this study was to evaluate incremental inclusion levels (0, 12, 24 and 36%) of dried and coarsely ground *Opuntia* cladodes in four complete and balanced diets for sheep. Based on the inclusion levels of the dried and coarsely ground *Opuntia* cladodes, the treatment diets were designated T0, T12, T24 and T36. The composition of the four treatment diets is presented in Table 2.1.

Table 2.1 The composition of the four treatment diets with incremental inclusion levels of dried and coarsely ground *Opuntia* cladodes

	Treatment diets			
Feeds	T0	T12	T24	T36
Coarsely ground *Opuntia* cladodes (kg)	0	120	240	360
Coarsely ground lucerne hay (kg)	660	535	410	285
Yellow maize meal (kg)	300	300	300	300
Feed grade urea (kg)	0	5	10	15
Molasses meal (Calori 3000) (kg)	40	40	40	40

Before the four diets were mixed, the lucerne hay was also ground to pass through the 20 mm sieve in the small hammer mill. The other feeds used in the diets, namely the yellow maize meal, feed grade urea and molasses meal, were included in the physical form in which these feeds were purchased. This decision was deliberate with a view to formulate and constitute diets that could be applied with a minimum of further processing and with direct application at the farm level. Because of the small quantities of feeds required in this study, the four trial diets were thoroughly mixed with a garden spade on a dry, clean cement floor.

2.1.2 Experimental animals

Twenty four young Dorper wethers, with an average body weight of 36.0±3.49 kg were used to evaluate the four diets. The 24 Dorper wethers were stratified according to body weight and allocated in four groups of six to each of the treatment diets T0, T12, T24 and T36, respectively.

The six wethers in each of the four treatments were housed outdoors as separate groups in small kraals. After the wethers have been weighed on 9 June 2004 they were adapted to their respective diets over a period of nine (9) days: after an initial period of five (5) days of adaptation outdoors, they were weighed again on 14 June 2004 and randomly housed in 24 individual metabolism crates for the last four (4) days of adaptation to the diets and especially also to the new environment of being housed in metabolism crates. The feed intake and digestibility trial commenced on 18 June 2004 and lasted 10 days until 27 June 2004. The wethers were then removed from the metabolism crates, weighed and housed outdoors again where they were fed a common diet of coarsely ground lucerne hay.

The individual metabolism crates were designed specifically to separate and collect the faecal and urine excretion of male sheep separately. In addition, the design of the metabolism crates was such that the daily feed and water intake of each sheep could be determined. The metabolism crates were designed to prevent the sheep from turning around; they could only face towards the feed and water troughs, thus contamination of the feed or water with faeces were limited.

2.1.3 Trial procedures

2.1.3.1 Feeds

The total feed intake of the four treatment groups of six Dorper wethers was noted during their adaptation period; because they were accommodated in groups feed intake could not be measured individually. Once the wethers were housed in the metabolism crates, they were offered food at a 10% refusal level of intake, calculated on a daily basis by using a 3-day moving average feed intake of the preceding three days. The feed offered and feed refusals of the wethers were measured in 24-hour cycles, starting every day at noon. The feed refusals were weighed back and then the quantity of feed to be fed to each wether during the following 24-hour cycle was calculated and weighed into large, brown paper bags. Approximately half of the feed weighed in this way was given to each wether after it was weighed at the start of each 24-hour cycle. The remaining feed was then given in two portions, namely at 16h30 the afternoon and 08h30 the following morning. In the event that a wether ate all its weighed feed before the end of a 24-hour cycle, more feed was weighed, recorded and provided to that specific wether.

About three days after the 9-day adaptation period commenced most of the wethers in treatments T24 and T36 began to produce wet faeces, reminiscent of diarrhoea. Therefore, a prophylactic dose of about 0.7 g Kaolin (Hydrated aluminum silicate, Al_2O_3 $2SiO_n$ $2H_2O$) was given to each wether at every feeding in an attempt to prevent or limit the excretion of wet faeces. Although this dietary prophylactic measure had no clear visible effect on the animals that were excreting very wet faeces, routine prophylactic treatment with 0.7 g Kaolin per wether per feeding was continued for the duration of the trial.

The feed refused by each wether for the duration of the 10-day trial period were collected and dried in a force draught oven at 100°C for at least 16 hours. After thorough mixing, representative samples were taken from the pooled feed refusals of each wether, ground to pass through a 1 mm sieve and stored pending chemical analysis. A composite feed sample from each treatment diet offered was collected on a daily basis for the duration of the trial. The composite or pooled sample from each treatment diet was dried to determine the dry matter (DM) content, then ground to pass through a 1 mm sieve and stored pending chemical analysis.

2.1.3.2 Water

Plastic buckets with a volume of about 5 *l* were used to provide water *ad lib.* to the wethers in the metabolism crates. The buckets were filled with 4 *l* water at the start of the trial period. The water level in each bucket was checked twice a day relative to a calibrated marker at the side of the bucket, before the sheep were fed in the mornings and afternoons. A measured quantity of water was added and recorded when required. The buckets were emptied and cleaned at least every three days to prevent the feed that fell into the water from fouling the water, making it unacceptable to the sheep. Water intake was measured by subtracting the volume of water remaining in a bucket at the time when it was cleaned. No allowance was made for evaporation loss of water indoors from the buckets.

2.1.3.3 Faeces

The faeces of each sheep was collected daily in separate large, brown paper bags and weighed before being placed in a force draught oven at 100°C for at least 48 hours or until assessed to be dry. However, some of the faecal material took a much longer time to dry because it was very wet. The wet faeces formed a crust once it started drying in the oven that impeded the drying process. This meant that these faeces had to be left in the drying oven for about 72 hours or even longer while any crusts that formed were broken when noticed. The faeces that were excreted in the more usual form of small sheep pellets dried quicker and could therefore be removed from the oven after about 48 hours.

The DM was calculated by weighing the faeces after it was dried as described above. After thorough mixing of the total faecal excretion, a representative sample from each sheep's faeces was taken and ground to pass a 1 mm sieve. The ground faeces were stored in plastic jars with airtight screw tops pending chemical analysis.

2.1.3.4 Urine

Urine was collected in dark, brown glass bottles. The urine was collected from the base of the metabolism crates and directed via urine collection plates to the bottles; a funnel protected with a sieve was inserted in each bottle. The funnels and bottles were placed directly under the urine collection plates of the metabolism crates so that all urine would pass through the sieves and funnels. However, due to the wet nature of some of the faeces, the urine of a number of the wethers was apparently more contaminated than would normally be expected which could have affected among others the N content of the urine.

A preservation solution (4N H_2SO_4 with 9% $CuSO_4$) was added to each bottle at an inclusion level of 5% to prevent microbial activity (De Waal, 1979) and volatilisation of ammonia from urine (AOAC, 2000). Urine levels in the bottles were checked daily after each feeding.

When a bottle was filled close to capacity, an exact quantity of 2 *l* urine was decanted and recorded; 10 ml of the preservation medium was then added to the bottle again.

2.1.4 Chemical analysis

2.1.4.1 General

The samples used for the different laboratory analyses, were dried overnight in a force draught oven at 100°C. The dry samples were then removed from the oven and cooled in desiccators before being weighed and introduced into the different analytical procedures. All analyses were therefore conducted on a DM basis.

2.1.4.2 Dry matter (DM)

The faeces, feed refusals and composite feed samples collected during the collection period of 10 days were weighed and then dried in a force draught oven at 100°C for at least 24 hours. As mentioned previously (see 2.1.3.3), the faeces of some wethers were quite wet and were left in the oven for longer periods to dry. After being assessed as dry and no further weight (water) loss occurred, the faeces were removed from the oven and weighed to determine the DM content.

The DM content of the faeces, feed refusals and feed samples was calculated as follows:

$$\text{DM (g/kg)} = \frac{\text{Weight of sample (g) after drying}}{\text{Weight of sample (g) before drying}} \times 1000$$

2.1.4.3 Organic matter (OM)

Samples of approximately 2 g were weighed accurately into dry, previously weighed porcelain crucibles to determine the ash content or conversely the organic matter (OM) content. The crucibles plus samples were then dried overnight at 100°C in a force draught oven. The crucibles plus samples were then cooled in desiccators and weighed. The crucibles plus samples were then placed in a muffle furnace and incinerated for 4 hours at 550°C

(AOAC, 2000). Crucibles plus ash were then cooled in desiccators and weighed again. The OM analyses were done in duplicate.

The OM content of samples was determined as follows:

$$\text{OM (g/kg)} = \frac{\text{Weight of sample (g)} - \text{weight of ash (g)}}{\text{Weight of sample (g)}} \times 1000$$

2.1.4.4 Crude protein (CP)

Approximately 0.2 g DM of each sample was weighed accurately in a tiny foil cup. The foil cups plus samples were then inserted in a Leco® Nitrogen analyser (Leco® Corporation, 2001) and the total nitrogen (N) content determined on combustion in oxygen. A factor of 6.25 was used to convert the N content of the samples to crude protein (CP) content. All N analyses were done in duplicate.

2.1.4.5 Ether extract (EE)

Samples of approximately 2 g were weighed accurately and folded into filter paper before being inserted into a cellulose extraction bullet to determine the ether extract (EE) content (AOAC, 2000). Cotton wool was placed in the top of the extraction bullets to prevent washing out of the samples. Bullets were then placed in a Soxhlet apparatus and extracted with hexane for 4 hours (S.W. van der Merwe, 2004; personal communication). The hexane extract was collected in flasks that were dried and weighed previously. After the extraction period, the remaining hexane was evaporated and the flasks placed in a force draught oven at 100°C to dry overnight. The flasks were then cooled in desiccators before being weighed again to determine the hexane soluble fraction. All EE analyses were done in triplicate.

The EE fraction of samples was determined as follows:

$$\text{EE (g/kg)} = \frac{[\text{Flask weight (g)} + \text{Ether extract}] - \text{Flask weight (g)}}{\text{Sample weight (g)}} \times 1000$$

2.1.4.6 Acid-detergent fibre (ADF)

The acid-detergent fibre (ADF) content was determined according to the procedures described by Goering and Van Soest (1970) and Robertson and Van Soest (1981). Samples of approximately 1 g were weighed accurately and placed in sinter glass crucibles. The crucibles were then placed in a Tecator Fibertec System M 1020 Hot extractor for analysis. After ADF solution was added to the samples it was boiled for an hour. After this the solution was drained and the samples rinsed with boiling water and then with acetone. Samples were placed in a force draught oven to dry overnight at 100°C. Dry samples were then removed from the oven and allowed to cool in desiccators. The samples were then weighed again before being placed in a muffle furnace and incinerated for 4 hours at 550°C. The samples were then removed from the furnace, allowed to cool in desiccators and weighed again.

The ADF content of samples was calculated as follows:

$$\text{ADF (g/kg)} = \frac{[\text{Sample weight (g)} - \text{Sample weight after boiling (g)}] - \text{Ash weight (g)}}{\text{Sample weight (g)}} \text{ x } 1000$$

2.1.4.7 Neutral-detergent fibre (NDF)

The neutral-detergent fibre (NDF) content was determined according to the procedures described by Goering and Van Soest (1970) and Robertson and Van Soest (1981). Samples of approximately 1 g were weighed accurately and placed in sinter glass crucibles. The crucibles were then placed in a Tecator Fibertec System M 1020 Hot extractor for analysis. After NDF solution was added to the samples it was boiled for an hour. The solution was then drained and the sample was rinsed with boiling water and then with acetone. Samples were placed in a force draught oven to dry overnight at 100°C. Dry samples were then removed from the oven and allowed to cool in desiccators. The samples were then weighed again before being placed in a muffle furnace and incinerated for 4 hours at 550°C. The samples were removed from the furnace, allowed to cool in desiccators and weighed again.

The NDF content of samples was calculated as follows:

$$\text{NDF (g/kg)} = \frac{[\text{Sample weight (g)} - \text{Sample weight after boiling (g)}] - \text{Ash weight (g)}}{\text{Sample weight (g)}} \times 1000$$

2.1.4.8 Gross energy (GE)

The gross energy (GE) of the four treatment diets and the feed refusals and faeces of the 24 wethers was determined on a DM basis by using an adiabatic bomb calorie meter (AOAC, 2000).

2.1.4.9 Apparent digestibility coefficients

According to McDonald *et al.* (2002) the apparent digestibility of feed or nutrients is best defined as the proportion of ingested feed or nutrients not excreted in the faeces and therefore assumed to be absorbed by the animal.

The following formula was used to calculate apparent digestibility coefficients:

$$\text{Apparent digestibility coefficient} = \frac{\text{(feed or nutrient intake) - (feed or nutrient excreted in faeces)}}{\text{Feed or nutrient intake}}$$

where Intake (kg) = kg feed or nutrient presented - kg feed or nutrient refused.

Note that the apparent digestibilities are presented in this study as coefficients and not as percentages.

2.2 Feed intake, digestibility and rumen fermentation trial

2.2.1 Preparation of feeds

A second batch of 873.5 kg freshly pruned *Opuntia* cladodes was collected on 3 Augustus 2004 according to the same procedures as previously described (see 2.1.1). This dried and coarsely ground *Opuntia* cladodes was used to augment the remainder of the four diets used in the first trial to conduct the second trial for the evaluation of rumen variables. However, the procedure to cut the *Opuntia* cladodes for drying in the sun was modified and greatly improved. At the research facility the *Opuntia* cladodes were cut into narrow strips of about 10 to 15 mm with an improvised implement that was designed to cut the cladodes more effectively and quicker than was possible with a long bladed butchers' knife. The design of the improvised implement is based on the mechanism of a paper guillotine with a sharpened machete serving as the cutting blade. It proved to be very effective, being faster and easier to use than cutting *Opuntia* cladodes strips by hand with a knife. It enables the operator to cut thinner strips, facilitating more effective drying. The strips were once again placed outdoors to dry for a week. The same procedures as described previously to dry and ground the *Opuntia* cladodes were followed (see 2.1.1).

The dried and coarsely ground *Opuntia* cladodes were then used to mix another four batches of the diets (Table 2.1; T0, T12, T24 and T36) in the same way as described previously (see 2.1.1). These four new batches were added to and thoroughly mixed with the respective remainder of the four treatment diets carried forward after the conclusion of the first trial.

2.2.2 Experimental animals

Four Dorper wethers were randomly recruited from the 24 wethers used in the first trial (see 2.1.2). The four wethers with an average weight of 36.0±2.44 kg were fitted with permanent rumen fistulae according to the technique described by De Waal *et al.* (1983). After the rubber rumen cannulae (25 mm inner diameter) were inserted to keep the permanent rumen fistulae open, the wethers were weighed again. The four rumen cannulated wethers were then randomly allocated to outdoor kraals (see 2.1.2) and each wether received a specific diet (see 2.2.1) according to a crossover design (Williams, 1949) as shown in Table 2.2.

The crossover design (Table 2.2) was constructed in such a way that each treatment (T0, T12, T24 and T36) comes before every other treatment the same number of times. This was to balance for carryover effects (Williams, 1949).

Table 2.2 The crossover design of the second trial with four rumen cannulated young Dorper wethers fed the four treatment diets (T0, T12, T24 and T36) with incremental inclusion levels of dried and coarsely ground *Opuntia* cladodes

	Rumen cannulated Dorper wethers			
	Wether 1	Wether 2	Wether 3	Wether 4
	Treatment diets			
Trial period A	T0	T12	T24	T36
Trial period B	T12	T36	T0	T24
Trial period C	T24	T0	T36	T12
Trial period D	T36	T24	T12	T0

The adaptation period on the specific diets (T0 or T12 or T24 or T36; see Table 2.2) that was allocated to each of the four rumen cannulated wethers, lasted one week (7 days). The wethers were fed twice daily and drinking water was provided *ad lib.* in water troughs. Daily feed intake of each of the four wethers was determined during the adaptation period of seven days. The feed refusals were collected on a daily basis for every wether and pooled. Feed samples of all four diets were collected continuously to determine DM content.

At the end of the 7-day adaptation period the four rumen cannulated wethers were weighed again and randomly housed individually in metabolism crates (see 2.1.2). The feed intake and digestibility of the four diets were then determined over a period of seven (7) days. During the last two (2) days of the feed intake and digestibility trial in the metabolism crates, namely on days 13 and 14 since the adaptation on a specific diet commenced for each of the four rumen cannulated wethers, the rumen variables were studied.

After the completion of the first trial period A on the four diets, the wethers were removed from the metabolism crates, weighed and moved outdoors to the open kraals again. Each wether was then changed over in a specific order to a new diet (see Table 2.2) and again adapted for a week on the new diet before being moved to the metabolism crates and a next trial period B of seven days commenced. The same procedures were applied for trial periods C and D respectively.

During this trial, each of the four rumen cannulated wethers were always kept in the same kraals outdoors and housed in the same metabolism crates for each of the four consecutive trial periods. Thus, it was attempted to reduce variation by subjecting the wethers only to a different diet (T0 or T12 or T24 or T36, respectively; see Table 2.2) at every crossover and minimise other undue stress as far as possible.

2.2.3 Feed intake and digestibility trials

2.2.3.1 Feeds

Feed intake and digestibility trials, similar to the one previously described (see 2.1.3) were performed consecutively during trial periods A, B, C and D. However, since only four rumen cannulated Dorper wethers were used to study the rumen variables in a crossover design, the procedures were slightly modified. The 24-hour cycles started at 08h00 in the morning in the second trial. The wethers were fed twice daily at 08h00 and again at 16h00. Feed refusals were collected each morning before feeding. Similar to the procedures previously described (see 2.1.3.1), the wethers were again fed at a 10% refusal level of intake, calculated on a daily basis by using a 3-day moving average feed intake of the preceding three days.

Feed refusals were collected daily for the duration of the seven days of the adaptation periods as well as the seven days of the feed intake and digestibility trials. The samples were pooled and dried overnight in a force draught oven at 100°C to determine the DM content. The dried samples collected during each of the seven day trial periods were then ground to pass through a 1 mm sieve and stored pending laboratory analysis.

All laboratory analyses were performed as previously described (see 2.1.4).

Samples of the four diets (T0, T12, T24 and T36) were collected daily and pooled in large, brown paper bags. At the conclusion of each of the four trial periods, the samples were weighed and dried overnight in a force draught oven at 100°C to determine DM content. The feed samples were then ground to pass a 1 mm sieve and stored pending laboratory analyses.

2.2.3.2 Water

As described previously (see 2.1.3.2) water was supplied *ad lib.* in 5 *l* plastic buckets to the wethers in the metabolism crates. The buckets were initially filled with 4 *l* of water. The water was checked and topped up every afternoon before feeding as required. The remaining water in each bucket was poured out and measured every morning; the buckets were then cleaned before fresh water was supplied. Total water intake of each wether was determined for the duration of each one-week trial period.

2.2.3.3 Faeces

The faeces of each wether was collected every morning after feeding and placed separately in large, brown paper bags. Each bag of faeces was then weighed and placed into a force draught oven at 100°C until assessed to be dry; in some cases this process took longer than normally anticipated (see 2.1.3.3). Total DM excreted was then determined. After thorough mixing of the total faecal excretion of each wether, representative samples from the pooled faeces of each wether were taken and ground to pass a 1 mm sieve for laboratory analysis.

2.2.3.4 Urine

Urine was collected in large, dark glass bottles as previously described (see 2.1.3.4). Urine excreted was measured regularly to determine the total volume excreted for the duration of each trial period.

2.2.4 Rumen fermentation

A major goal in this trial of the study was to evaluate the effect of incremental inclusion levels of dried and coarsely ground *Opuntia* cladodes in balanced diets for sheep on rumen fermentation, as reflected in specific rumen variables (De Waal *et al.*, 1989; De Waal, 1995).

2.2.4.1 *In sacco* dry matter (DM) disappearance in the rumen

Twelve nylon bags per wether (48 bags in total) with a pore size of 53 μm and an approximate size of 140 mm x 70 mm were placed in a force draught oven to dry overnight at 100°C according to the basic procedures described by De Waal (1995). The nylon bags were allowed to cool in desiccators and then weighed accurately. About 5 g of the air dry samples of each of the four diets (T0, T12, T24 and T36) were put into each of 12 previously weighed nylon bags (4 diets x 12 bags each = 48 bags). The nylon bags containing the feed samples were placed in a force draught oven to dry overnight at 100°C. The dry nylon bags containing the dry feed samples were then allowed to cool in a desiccator before being weighed again.

The feed samples were incubated in the nylon bags (*in sacco*) in the rumen of the four rumen cannulated wethers for periods of 0, 2, 4, 8, 12 and 24 hours respectively according to the basic procedures described by De Waal *et al.* (1989) and De Waal (1995). A maximum of only six bags per wether at one time was placed in its rumen to prevent overloading of the rumen with bags. The schedule designed in this trial to introduce and remove the nylon bags via the rumen cannulae was based on the procedures described by De Waal *et al.* (1989), namely:

Day 1: 08h00: six bags were placed via the rumen cannulae in the rumen of each wether. Another two bags, designated the **0 h** incubation sample, were not inserted into the rumen but immediately subjected to the washing procedure (see below).
10h00: two bags were removed from the rumen after **2 h** incubation.
20h00: two bags were removed from the rumen after **12 h** incubation.
Day 2: 08h00: the last two bags were removed from the rumen after **24 h** incubation and then the last four of the 12 nylon bags were placed in the rumen for incubation.
12h00: two bags were removed from the rumen after **4 h** incubation.
16h00: the last two bags were removed from the rumen after **8 h** incubation.

This procedure effectively covered the total 24-hour period without causing undue stress to the sheep because of too frequent handling to insert or collect samples via the rumen cannulae.

After the nylon bags were removed from the rumen they were placed directly into cold water from a tap to stop microbial action and then washed thoroughly until the wash water running from the bags was clear (De Waal *et al.*, 1989; De Waal, 1995). The nylon bags were then placed in a force draught oven to dry overnight at 100°C. After cooling in a desiccator, the nylon bags with the feed residues remaining after the respective incubation periods in the rumen described previously were weighed again and the quantity of feed material that disappeared was determined as follows:

In sacco disappearance =

[Bag plus feed before incubation (g) – Bag (g)] – [Bag plus residue after incubation (g) – Bag (g)]

The material remaining in the nylon bags after the respective incubation periods was used to determine the DM and NDF content according to the methods described in 2.1.4.2 and 2.1.4.7.

2.2.5 Collection of rumen fluid

Rumen fluid was collected at the same time that the nylon bags were placed in the rumen or removed from the rumen, *i.e.* after 0, 2, 4, 8, 12 and 24 hours of incubation in the rumen (see 2.2.4.1). The rumen fluid was aspirated via the cannulae with a flexible rubber tube connected to a 1 litre pliable plastic bottle (De Waal *et al.*, 1989). The plastic bottle was squeezed gently to create a partial vacuum that facilitated aspiration of the rumen content, both fluid and some solid material into the bottle. The rumen content was then strained through four layers of cheesecloth to remove the solid material and retain the strained rumen fluid for further processing and analysis.

2.2.5.1 Rumen pH

The pH of the rumen fluid was measured immediately with an electronic pH meter (HANNA Instruments Model 8521).

2.2.5.2 Rumen ammonia (NH_3) concentration

Approximately 100 ml of the strained rumen fluid samples was acidified with 1 ml concentrated H_2SO_4 in small plastic bottles with screw caps. The samples were then frozen pending analysis of NH_3 concentration by distillation over MgO according to procedures described by De Waal (1986) and De Waal *et al.* (1989).

In brief, these procedures (De Waal, 1986; De Waal *et al.,* 1989) comprise the following. Before analyses of NH_3 concentration commenced, a solution of 0.01 N H_2SO_4 was standardised with Borax. A saturated solution of boric acid was prepared and 120 ml indicator (2 parts methylene blue and 5 parts methylene red) was added to 10 *l* of the boric acid solution. For every analysis 50 ml of this boric acid/indicator solution was placed in a 250 ml Erlenmeyer flask.

Prior to analysis, the frozen rumen fluid samples were thawed overnight at room temperature in the laboratory. Each bottle was then shaken vigorously and the suspended solid material allowed to settle as a sediment before 20 ml of the supernatant was added to a large glass tube containing 50 ml distilled water, 3 glass beads and 0.4 ml octyl alcohol. Five teaspoons of MgO was added to the glass tube directly prior to it being placed in a Büchi 320 N Distilling Unit. The steam valve was opened and the solution containing the rumen fluid boiled until the Erlenmeyer flask containing the boric acid/indicator solution filled up to 250 ml with NH_3 containing distillate.

The NH_3 containing distillate was then titrated with the 0.01 N H_2SO_4 while stirred until a light purple colour was visible. The NH_3 concentration of the rumen fluid was determined as follows (De Waal, 1979; 1986):

$$\text{mg NH}_3 \text{ per 100 ml rumen fluid} = \frac{(0.14 \times \text{N of H}_2\text{SO}_4) \times \text{Titration value} \times 1.2143 \times 100}{\text{Sample size (ml)}}$$

Note that the NH_3 concentration in this study was expressed as mg NH_3 per 100 ml rumen fluid and not as NH_3-N concentration (De Waal *et al.*, 1989).

2.2.5.3 Rumen volatile fatty acid (VFS) concentration

Aliquots of 1 ml of the strained rumen fluid samples was put into small vials to which 0.25 ml of a 30% formic acid solution was added to stop any further microbial activity. These samples were then refrigerated at about 4°C pending volatile fatty acid (VFA) analysis.

Prior to analysis, the rumen fluid was centrifuged for 10 minutes at 3000g. The supernatant was removed and placed in a small, gas chromatography (GC) bottle for automatic injection and 1 µl was injected into the Gas Chromatograph (Hewlett Packard 5890A gas chromatograph with a Flame ionisation detector) for analysis.

Individual VFA concentrations were determined by using known external standards.

2.2.5.4 Statistical analysis

The data was statistically analysed using General Linear Models Procedures and the General Linear Models Procedures Repeated Measured Analysis of Variance of SAS (1991).

2.3 Processing and drying of *Opuntia* cladodes

It is a real challenge to dry *Opuntia* cladodes in the sun. Therefore, a separate pilot trial was conducted to get some indication of what would be a more effective method to dry the *Opuntia* cladode strips in direct sunlight.

By placing *Opuntia* cladode strips outside on wire mesh about 700 mm off the ground to promote air circulation, yielded the best results. By following the usual advice for drying namely to dry cut pieces of *Opuntia* cladodes on any clean surface such as a cement slab (Terblanche *et al.*, 1972; Steenkamp, 1973; Steenkamp & Hayward, 1981) caused the wet strips to mould or rot in places where air circulation was restricted. When placed on a wire mesh, air could circulate more freely around the cladode strips resulting in more effective drying. If the strips overlap they should be turned over frequently with a hay fork or other appropriate implement to expose all cut areas to adequate air flow.

Some form of protection should be provided against rain when necessary, because while drying, the *Opuntia* cladode strips will absorb rain water and slow down drying. Therefore, the *Opuntia* cladode strips can be placed under a roof, or appropriate plastic sheeting can be used to cover the strips and prevent dampening.

Maximum exposure to direct sunlight is recommended. When the *Opuntia* cladodes strips were cut lengthwise they seemed to dry quicker. More research is suggested on this subject to determine the ideal width of *Opuntia* cladode strips because, especially with young *Opuntia* cladodes, the strips seemed to dry only to a point if they are not thin enough. The strips curl up and the outer skin, as well as the callous layer that forms over the cut area, effectively prevents water loss and further drying. To dry the *Opuntia* cladodes to a point where it can be processed and included in diets it has to be ground coarsely, providing smaller uniform pieces with larger surface areas exposed to air for drying. More research is also suggested to determine if changes occur in the chemical composition during these different drying procedures. It was observed that when *Opuntia* cladode strips were dried indoors and thus protected from direct sunlight, it was greener in appearance when dry than those that were dried outside in direct sunlight. This might suggest some chemical change caused when exposed to direct sunlight.

When the uncut *Opuntia* cladodes were slightly dehydrated, *i.e.* storing it for about a week after being cut from the plants, the strips seemed to dry slightly faster. However, the problem when leaving the uncut *Opuntia* cladodes intact for a longer period of time they tend to bruise more easily when handled again. When stored in a heap for an extended period of time, they also start rotting. The rotten parts must then be discarded. The implication is that *Opuntia* cladodes can be cut in fairly large quantities during pruning operations in orchards and stored for a limited time in stacks while waiting to be cut in strips to dry. Careful handling is required though and the *Opuntia* cladodes should not be stacked high or kept in bags as this also causes bruising. Mondragón-Jacobo (2000) suggested stacking of cladodes on their sides in a shaded place.

When grounding the partially dried *Opuntia* cladode strips the material is still very sticky probably due to the mucilage content and tends to clog up the hammer mill and the sieve, especially if it is not quite dry as is the case after only a week of drying in the sun. The hammer mill therefore needs to be cleaned regularly and the material that has been coarsely ground needs to be dried further (see 2.1.1).

The *Opuntia* cladodes used in this trial was ground to pass through a 20 mm sieve. The material had to be ground a second time after a further drying period in the sun; this was to prevent the wethers from selecting from the treatment diets and leave some of the *Opuntia* cladode pieces when the material was coarser than about the size of a large pea.

3. Feed intake and digestibility trial

Cladodes of the spineless cactus pear *Opuntia ficus-indica* var. Algerian was used in this study and is referred to in an abbreviated format as *Opuntia* cladodes (see Chapter 2).

3.1 Diets

In the first trial of the study, 24 young Dorper wethers were used to determine the voluntary feed intake and digestibility of the four treatment diets (Table 2.1; T0, T12, T24 and T36) with incremental inclusion levels of dried and coarsely ground *Opuntia* cladodes.

3.1.1 Chemical composition of *Opuntia ficus-indica* var. Algerian cladodes

The chemical composition of the *Opuntia* cladodes used in the first trial of the study to determine the feed intake and digestibility of the four treatment diets is presented in Table 3.1.

Table 3.1 Chemical composition of *Opuntia ficus-indica* var. Algerian cladodes

	Opuntia ficus-indica var. Algerian
Dry matter (DM) (g/kg fresh cladodes)	99.0
Organic matter (OM) (g/kg DM)	806.1
Crude protein (CP) (g/kg DM)	84.0
Ether extract (EE) (g/kg DM)	16.6
Acid-detergent fibre (ADF) (g/kg DM)	168.7
Neutral-detergent fibre (NDF) (g/kg DM)	243.9
Gross energy (GE) (MJ/kg DM)	13.624

The DM content of the freshly pruned *Opuntia* cladodes was very low. This is in agreement with results published by Ben Salem *et al.* (1996), Sirohi *et al.* (1997), Nefzaoui and Ben Salem (2000), Ben Salem *et al.* (2002b) and Batista *et al.* (2003). These authors reported that cactus pear has a DM content varying from as little as 74 g/kg to as much as 150 g/kg on a fresh basis. However, this range of DM content is in sharp contrast with the 237 g DM/kg reported by McMillan *et al.* (2002) for *Opuntia* cladodes. The high water content or conversely low DM content of *Opuntia* cladodes makes it a bulky feed and, therefore, poses a real problem when it has to be transported over larger distances.

The OM content of the *Opuntia* cladodes used in this trial (Table 3.1) was within the range that was shown in several studies to vary from a low 710 g OM/kg DM to a high 900 g OM/kg DM (Ben Salem *et al.*, 1996; Nefzaoui & Ben Salem, 2000, Ben Salem *et al.*, 2002b; McMillan *et al.*, 2002; Batista *et al.*, 2003).

The CP content of the *Opuntia* cladodes(Table 3.1) was slightly higher than most values reported in literature. Values that vary from 14 g CP/kg DM to 77 g CP/kg DM were reported by Ben Salem *et al.* (1996), Nefzaoui and Ben Salem (2000), Ben Salem *et al.* (2002b) and Batista *et al.* (2003) for different cultivars. Pretorius *et al.* (1997) reported higher CP values ranging from 68.2 g CP/kg DM when *Opuntia* varieties were irrigated to as much as 101.3 g CP/kg DM when it was produced under dry land conditions. Pretorius *et al.* (1997) also reported that the CP of the Algerian variety was markedly higher than the other *Opuntia* varieties used in their trial. Potgieter (2002) also stated that the main criticism against cactus pear cladodes as animal feed is that its CP content is too low for maintenance, but recent studies have shown that the CP content of some varieties is more than 100 g CP/kg DM. Sirohi *et al.* (1997) also reported a CP content of 92 g/kg DM and stated that this level falls within the normal range of CP content. According to Claassens and Wessels (1997) the N content of the cactus pears is also increased when fertilised with N.

The EE content Table 3.1) of the *Opuntia* cladodes was low, namely 16.6 g/kg DM. This is in accordance with the lower end of the values ranging from 15.5 to 25 g/kg DM as reported by Terblanche (1971), Nefzaoui and Ben Salem (2000) and Batista *et al.* (2003).

The ADF content (Table 3.1) of the *Opuntia* cladodes used in this trial is lower than the 190 to 248 g ADF/kg DM reported by Sirohi *et al.* (1997) and Batista *et al.* (2003). It is, however, higher than the 129 g ADF/kg DM reported by Ben Salem *et al.* (1996). The ADF content reported by Nefzaoui and Ben Salem (2000) varies considerably from 112 to 190 g ADF/kg DM.

Sirohi *et al.* (1997) and Nefzaoui and Ben Salem (2000) reported NDF contents of 312 to 400 g/kg DM for cactus pears, which is markedly higher than the NDF value in this trial (Table 3.1). The NDF content of the *Opuntia* cladodes used in this trial is, however, consistent with the range of 185 to 235 g NDF/kg DM reported by Ben Salem *et al.* (1996) and Ben Salem *et al.* (2002b).

The gross energy (GE) content (Table 3.1) of the *Opuntia* cladodes was lower than expected. According to McDonald *et al.* (2002), the average GE value for feeds is about 18.5 MJ/kg DM. Furthermore, feeds with a high ash content are much lower than average because the minerals do not contribute to the calorific content. The low OM content of the *Opuntia* cladodes ggests that the material may have alower than average GE content. Since very few studies seem to have determined and reported the GE of *Opuntia* cladodes, the paucity of information makes any meaningful comparison difficult.

The results shown in Table 3.1 reflect the chemical composition of *Opuntia* cladodes when pruned from plants in mid May 2004 (see 2.1.1); it may be different during other periods or seasons of the year. Walters (1951) and Retamal *et al.* (1987) reported seasonal variation in the chemical composition of cactus pears. According to Walters (1951) and Nefzaoui and Ben Salem (2000), the water content of the cactus pear plant is higher in the dry winter months (winter designated by Walters as being April to September in South Africa) than in the summer. Walters (1951) also reported that the CP, EE, ash and crude fibre (CF) contents of cactus pear plants increase on a DM basis in winter while the nitrogen free extract (NFE) content increases in summer.

According to Inglese (1995) cactus pears should not be pruned during the rainy or cold seasons but the pruning in orchards should be done when the temperature is high enough to allow the fresh wounds where the cladodes were cut from the trees, to dry quickly. According to Wessels (1988) the best time for pruning in South Africa is from May to July, after the fruits have been harvested. Therefore, this is the time of the year when large quantities of plant material harvested during pruning in orchards will be available for use in diets for animals.

3.1.2 Chemical composition of the four treatment diets

The chemical composition of the four treatment diets (T0, T12, T24 and T36) with incremental inclusion levels of dried and coarsely ground *Opuntia* cladodes, is presented in Table 3.2.

Table 3.2 Chemical composition of the four treatment diets with incremental inclusion levels of dried and coarsely ground *Opuntia* cladodes

	Treatment diets			
	T0	T12	T24	T36
DM (g DM/kg feed)	905.5	904.3	886.1	881.7
OM (g/kg DM)	904.6	885.0	890.0	869.3
CP (g/kg DM)	167.9	180.9	165.4	168.0
EE (g/kg DM)	23.5	24.9	25.8	27.0
ADF (g/kg DM)	304.0	265.6	239.2	207.2
NDF (g/kg DM)	470.8	410.2	410.0	315.0
GE (MJ/kg DM)	23.095	21.628	17.020	17.050

The DM content of the four diets (Table 3.2) decreased with the increasing inclusion levels of *Opuntia* cladodes. Cactus pears are succulent plants that do not dry easily (see 2.1.1). This is ascribed to the mucilage content, a hydrophilic mucus-like compound that has a high water-holding capacity (Cárdenas *et al.*, 1997; Tegegne, 2002; Sáenz *et al.*, 2004). The precise function of the mucilage is not known, however, it is generally believed that it helps to retain water inside the cactus (Mindt *et al.*, 1975; Sudzuki Hills, 1995). Therefore, with the 24% and 36% inclusion levels of the relatively dry and coarsely ground *Opuntia* cladodes in the diets, it appeared that the mucilage content have contributed to the retention of water in the *Opuntia* cladodes and thus played a role in the lower DM content of these diets.

The OM contents of the four diets (Table 3.2) deceased with incremental inclusion levels of *Opuntia* cladodes. The mineral content of cactus pear cladodes is known to be high (Batista *et al.*, 2003). In the present study the *Opuntia* cladodes contained 193.9 g ash/kg DM or conversely 806.1 g OM/kg DM. The specific minerals in the ash fraction of the *Opuntia* cladodes were not analysed, but it has been reported that cactus pears contain high levels of Ca (Batista *et al.*, 2003) and to a lesser extent also relatively high K levels (Ben Salem & Nefzaoui, 2002).

As discussed previously (see 3.1.1), the *Opuntia* cladodes used in this trial contained 84.0 g CP/kg DM, which is higher than the CP content of cactus pear cladodes reported in several studies (Ben Salem *et al.*, 2002b; Tegegne, 2002; Batista *et al.*, 2003). The CP content of the lucerne hay used in this study was 209 g CP/kg DM. Feed grade urea was used to balance the CP content of the four diets at a level of about 165 to 170 g CP/kg. Diet T12 (Table 3.2) had a slightly higher CP level than the other three diets, which was not expected. This might in part be ascribed to the substitution of the lucerne hay with its relatively high CP content (209 g CP/kg DM) with the *Opuntia* cladodes which has a relatively low CP content (84.0 g CP/kg DM), as well as the addition of only a very small quantity of feed grade urea, even at the highest level of inclusion (see Table 2.1). The decrease in the CP content of the diets derived from the lucerne hay as a result of the increasing levels of substitution with *Opuntia* cladodes in diets T24 and T36 was countered or compensated for by the addition of higher levels of urea, resulting in not much difference in the CP contents of diets T0, T24 and T36.

The EE content (Table 3.2) of the four diets increased slightly with the higher inclusion levels of dried and coarsely ground *Opuntia* cladodes. Not much increase in the EE content of the four diets was expected given the low EE content of the *Opuntia* cladodes (Table 3.1).

The ADF and NDF contents of the four diets decreased with the increasing inclusion levels of *Opuntia* cladodes (Table 3.2), clearly reflecting the low fibre content of *Opuntia* cladodes used in this trial, namely 168.7 g ADF/kg DM and 243.9 g NDF/kg DM (Table 3.1). These values are appreciably higher than the average 117 g NDF/kg DM and 28 g ADF/kg DM for maize and lower than the average 493 g ADF/kg DM and 375 g NDF/kg DM for lucerne hay (McDonald *et al.*, 2002).

The GE of the four diets (Table 3.3) decreased markedly with increasing inclusion levels of *Opuntia* cladodes up to 24% and thereafter no further decrease in GE was detected.

3.1.3 Water intake and urine excretion

The *Opuntia* cladodes were dried and coarsely ground when it was included in three of the treatment diets (T12, T24 and T36) to substitute coarsely ground lucerne hay (see Table 2.1). The dried *Opuntia* cladodes used in this trial thus contributed much less water than in most trials where it was fed in the fresh or partially dried form with high water content (Terblanche *et al.*, 1971; Ben Salem *et al.*, 1996). This may have affected both voluntary water consumption and urine excretion.

The daily water intake and urine excreted by the young Dorper wethers fed the four treatment diets are presented in Table 3.3.

Table 3.3 Daily water intake and urine excretion by the young Dorper wethers fed the four treatment diets with incremental levels of dried and coarsely ground *Opuntia* cladodes

	Treatment diets			
	T0	T12	T24	T36
Water intake (ml/day)	2235.5±190.6^{b}	2695.3±595.5^{a,b}	2949.3±592.0^{a,b}	3189.3±775.6^{a}
Urine excreted (ml/day)	779.1±150.3^{a}	811.8±212.4^{a}	844.8±160.2^{a}	949.7±191.4^{a}

^{a,b} Means in the same line with different superscripts differ significantly ($P<0.05$).

The daily voluntary water intake of the wethers (Table 3.3) increased significantly ($P<0.05$) with increasing inclusion levels of dried and coarsely ground *Opuntia* cladodes in the diets, but the differences in urine excretion were, however, not statistically significant ($P>0.05$). This may be ascribed to the fact that the *Opuntia* cladodes contain a complex carbohydrate, mucilage, with a great capacity to absorb water (Cárdenas *et al.*, 1997; Tegegne, 2002b; Sáenz *et al.*, 2004). As discussed previously, the precise function of the mucilage is not known, however, it is generally believed that it helps to retain water inside the cactus (Mindt *et al.*, 1975; Sudzuki Hills, 1995). Since mucilage binds strongly to water, it is quite plausible that this may render some water in the digestive tract of the wethers unavailable for absorption (see 3.1.2). Hence, the sheep may have needed to drink more water. The wethers on diet T36 with the highest inclusion level of *Opuntia* cladodes drank on average about 900 ml water per day more than the wethers on diet T0, but they only excreted on average about

171 ml urine per day more than those on diet T0. Since the voluntary water intake of the wethers was significantly ($P<0.05$) higher on diet T36 with the highest inclusion level of *Opuntia* cladodes, it would have been expected that the water excreted in their faeces would also be markedly higher, which was not the case.

3.1.4 Feed intake and faeces excreted

The daily feed intake and faeces excreted by the young Dorper wethers in the different treatments are presented in Table 3.4.

Table 3.4 Feed intake and faeces excreted by the young Dorper wethers fed the four treatment diets with incremental levels of dried and coarsely ground *Opuntia* cladodes

	Treatment diets			
	T0	T12	T24	T36
Feed intake (g DM/day)	1148.3±66.2^{a}	1119.2±153.5^{a}	1104.3±118.8^{a}	1085.9±162.7^{a}
Faeces excreted (g DM/day)	375.9±22.6^{a}	365.8±61.2^{a}	343.1±41.5^{a}	308.1±50.9^{a}
DM intake (g/kg $W^{0.75}$/day)	78.4±6.5^{a}	75.1±9.2^{a}	73.8±7.3^{a}	73.9±8.9^{a}

^{a,b} Means in the same line with different superscripts differ significantly ($P<0.05$).

The feed intake by the wethers decreased slightly (Table 3.4) with increasing levels of *Opuntia* cladodes in the diets, but these decreases were not statistically significant ($P>0.05$). Similarly, by expressing feed intake per metabolic weight (g/kg $W^{0.75}$/day), the differences were not statistically significant ($P>0.05$). This suggests that incremental inclusion levels of the dried and coarsely ground *Opuntia* cladodes up to a level of 36% in the diet did not markedly change the acceptability or palatability of the diets for young Dorper wethers.

The DM excreted in the faeces by the wethers on the four treatment diets (Table 3.4) decreased slightly but the decreases were not significantly ($P>0.05$) different. This suggests that, since the DM intake of the wethers and the DM excreted in their faeces did not differ significantly ($P>0.05$) between treatments, the four diets were utilised with the same apparent efficiency.

However, there were obvious differences in the consistency and appearance of the faeces excreted by the wethers on the four treatment diets as shown in Plates 1 to 4. Although some variation occurred among the six wethers within specific treatments, the faeces that emanated from diets T12 (Plate 2), T24 (Plate 3) and T36 (Plate 4) were much wetter than those from diet T0 (Plate 1).

Table 3.5 Body weight changes of the young Dorper wethers fed the four treatment diets with incremental levels of dried and coarsely ground *Opuntia* cladodes

	Treatment diets			
Body weight changes (kg)	T0	T12	T24	T36
Adaptation period (9 days)	0.100	0.0	-0.933	-0.900
Trial period (10 days)	0.500	0.533	1.433	1.650
Total trial (19 days)	0.600	0.533	0.500	0.750

The total body weight changes of the wethers from the beginning of the adaptation period until the conclusion of the trial after 19 days (Table 3.5) are not much different, suggesting that the overall effects of the diets on the wethers were small. Apparently the wethers took longer to adapt to those diets containing the higher incremental levels of dried and coarsely ground *Opuntia* cladodes, therefore, the wethers on diets T24 and T36 lost on average about 0.900 kg body weight during the adaptation period of nine days. The wethers on diet T0 gained slightly in weight (0.100 kg), whilst the wethers on diet T12 retained their body weight. During the following feed intake and digestibility trial period of 10 days, while confined to the metabolism crates, the wethers on diets T24 and T36 gained much more body weight than the wethers on diets T0 and T12, while there was little difference in their feed intake (Table 3.4). This suggests that the three diets containing the incremental levels of dried and coarsely ground *Opuntia* cladodes have been well utilised by the young Dorper wethers once they have adapted to it.

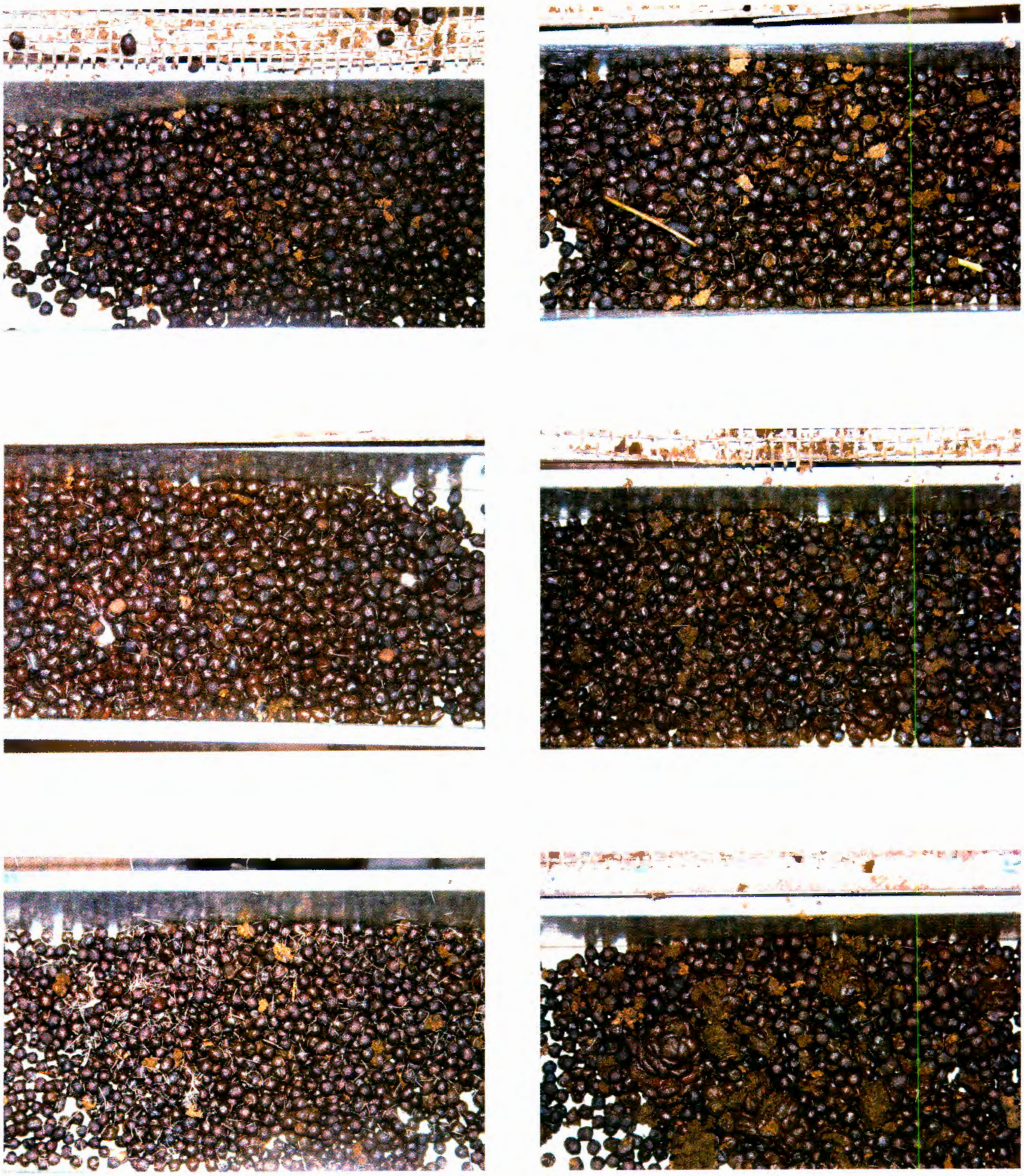

Plate 3.1 Faeces of six sheep on treatment diet T0

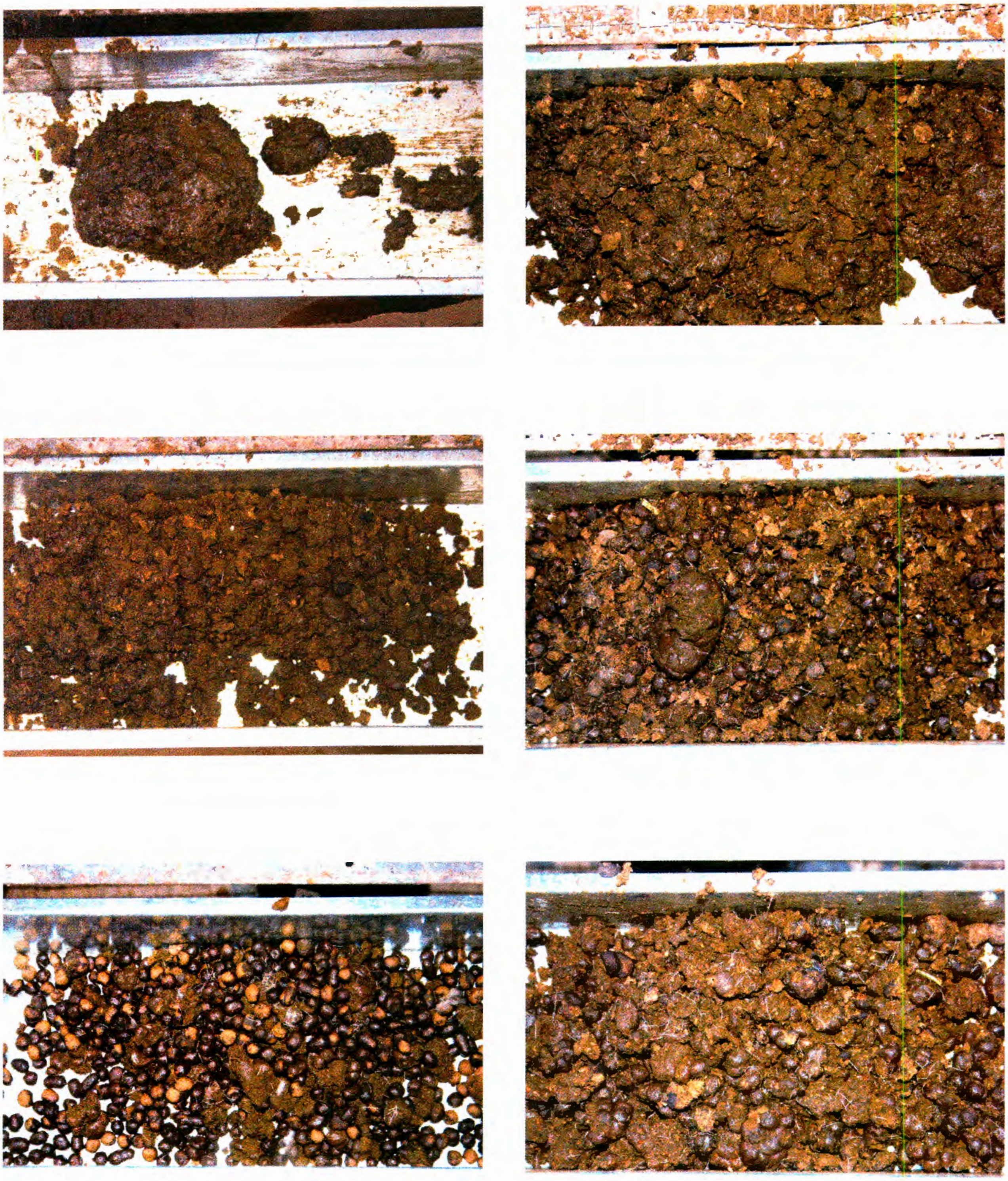

Plate 3.2 Faeces of six sheep on treatment diet T12

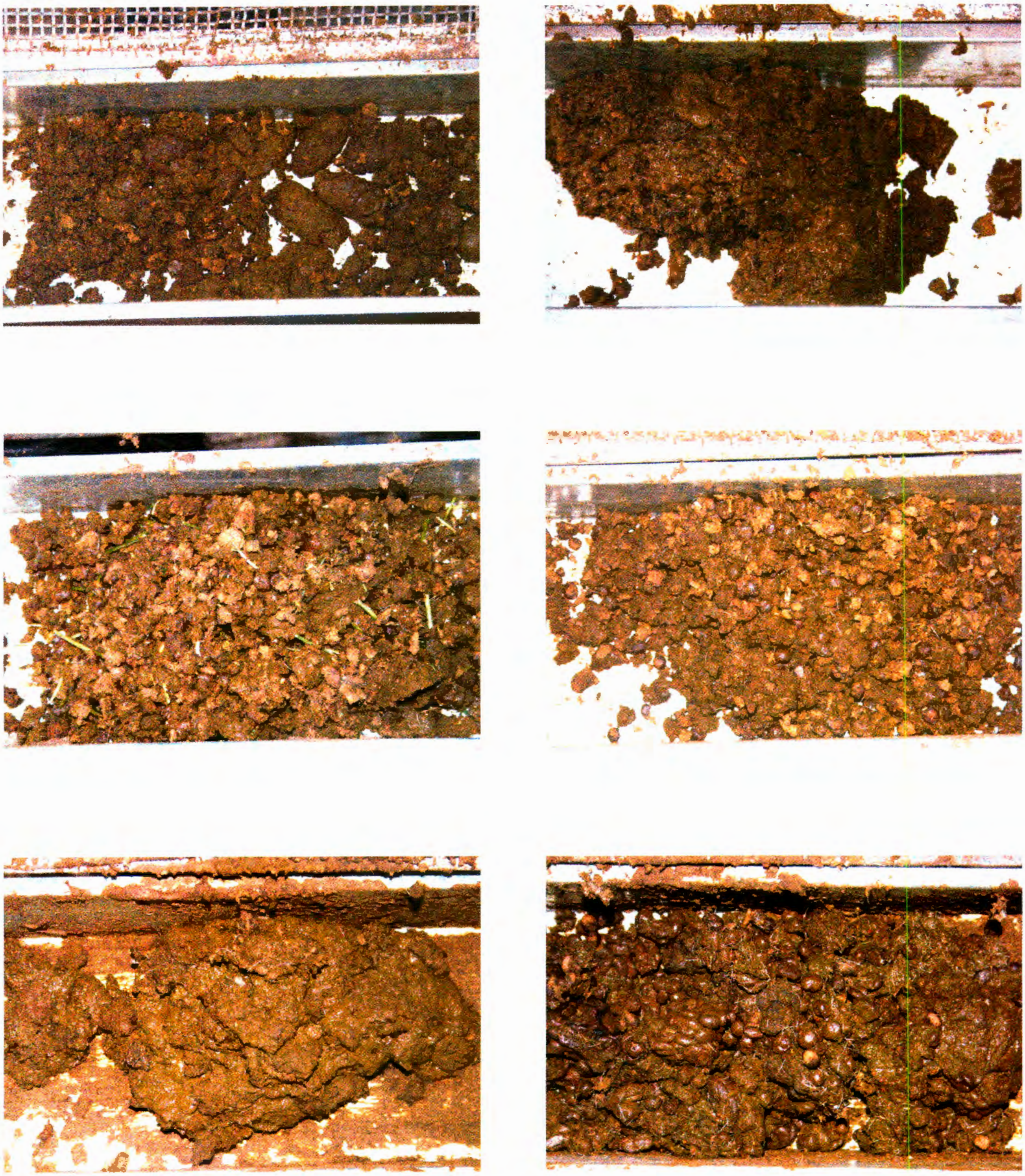

Plate 3.3 Faeces of six sheep on treatment diet T24

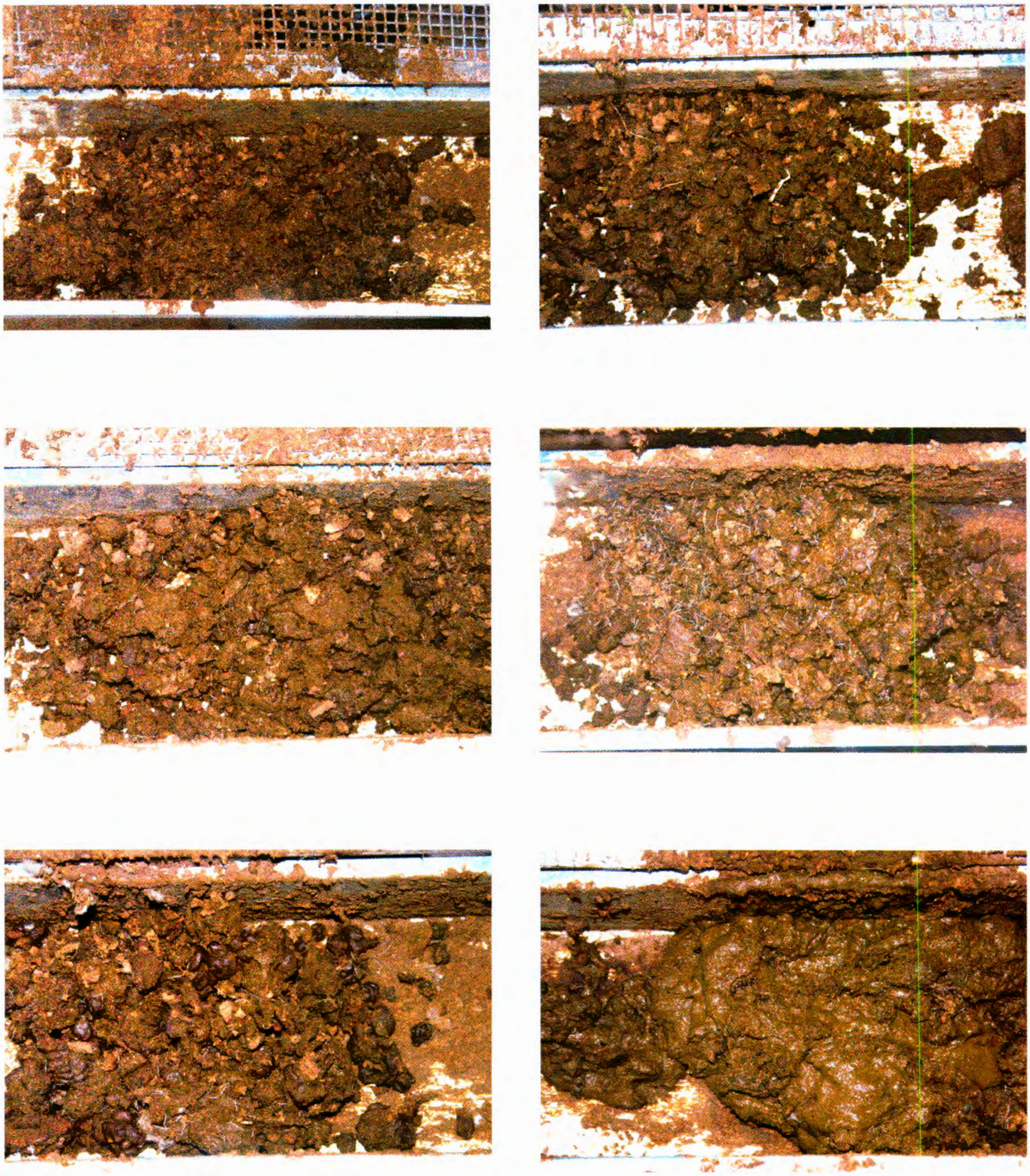

Plate 3.4 Faeces of six sheep on treatment diet T36

3.2 Chemical composition of the diets, feed refusals and faeces

3.2.1 Dry matter (DM)

The DM content of the four treatment diets and the DM content of the feed refusals and the faeces excreted by the young Dorper wethers in the four treatments are presented in Table 3.6.

Table 3.6 The DM content of the diets, feed refusals and faeces excreted by the young Dorper wethers fed the four treatment diets with incremental levels of dried and coarsely ground *Opuntia* cladodes

	Treatment diets			
	T0	T12	T24	T36
Diet (g DM/kg feed)	905.5	904.3	886.1	881.7
Feed refused (g DM/kg)	889.0±24.5^{a}	908.4±17.7^{a}	893.9±36.8^{a}	887.4±33.3^{a}
Faeces excreted (g DM/kg)	439.3±40.7^{a}	326.5±67.3^{b}	272.6±41.8^{b,c}	218.3±21.2^{c}

^{a,b,c} Means in the same line with different superscripts differ significantly ($P<0.05$).

The DM content of the four treatment diets has been discussed previously (see 3.1.1; 3.1.2; and Table 3.2). In Table 3.6 it is shown that the DM content of the diets and the feed refusals were not significantly ($P<0.05$) different. This suggests that the diet selection by the wethers from the four diets as reflected in the DM content was negligible.

The decrease in DM content of the faeces (Table 3.6) with incremental inclusion levels of dried and coarsely ground *Opuntia* cladodes in the diet was significant ($P<0.05$). This decrease in DM content or conversely the increase in water content of the faeces coincided with the higher water intake commensurate with higher *Opuntia* cladodes inclusion levels in the diets (Tables 3.3 and 3.4). The DM content of the faeces excreted by the wethers fed diet T0 was about twice that of those fed diet T36, or conversely the faeces was twice as wet.

According to Steenkamp (1973) the high magnesium (Mg) content of spineless cactus pears might explain the laxative effect when fed to ruminants. Nefzaoui and Ben Salem (2000) reported that the total oxalate content of cactus pear cladodes is about 130 g/kg DM from which about 40% is in a soluble form. They suggested that the oxalates are probably bound to Ca making this anion less available to animals. This high amount of oxalates may also explain the laxative effect of cactus pear cladodes when fed to animals (Nefzaoui & Ben Salem, 2000). According to Tegegne (2002b) very wet faeces is to be expected because of the high water and mucilage content of the cactus cladodes. However, results of this trial with young Dorper wethers suggested that it was not the inherent high water content of the *Opuntia* cladodes that caused the high water content of the faeces because the *Opuntia* cladodes were dried before inclusion into the diets. Therefore, it is more plausible that this phenomenon of wet faeces is attributed either to the high mucilage content and the great capacity of this hydrocolloid to imbibe water (Sáenz *et al.*, 2004) or the high oxalate content of cactus pears (Nefzaoui & Ben Salem, 2000). It should be noted that several reports showed a marked decrease in water consumption by sheep with increasing intake of fresh cactus pear cladodes with its high water content (Terblanche *et al.*, 1971; Ben Salem *et al.*, 1996); at the highest levels of cactus pear intake, the water consumption by the sheep even stopped (Ben Salem *et al.*, 1996). In this trial the sheep drank more water with increasing dietary inclusion levels of dried *Opuntia* cladodes (Table 3.3).

3.2.2 Organic matter (OM)

The OM content of the four treatment diets and the OM contents of the feed refusals and the faeces excreted by the young Dorper wethers in the four treatments are presented in Table 3.7.

The OM content of the four treatment diets was consistently higher than the OM content of the corresponding feed refusals (Table 3.7). This suggests that the wethers did some selection for feed ingredients from the diets but similar to the DM, the selection as reflected in the OM content was negligible. The differences in OM content of the feed refusals were not significant (P>0.05). The OM content of the faeces on diets T0 and T36 differed significantly from each other as well as from the other two diets (T12 and T24). The OM content of the faeces on diets T12 and T24 did not differ significantly (P>0.05) from each other.

Table 3.7 The OM content of the diets, feed refusals and faeces excreted by the young Dorper wethers fed the four treatment diets with incremental levels of dried and coarsely ground *Opuntia* cladodes

	Treatment diets			
	T0	T12	T24	T36
Diet (g OM/kg DM)	904.6	885.0	890.0	869.3
Feed refused (g OM/kg DM)	876.2±35.2^{a}	808.8±54.4^{a}	849.9±19.4^{a}	843.1±26.1^{a}
Faeces excreted (g OM/kg DM)	809.1±7.4^{a}	775.0±5.8^{b}	774.4±9.2^{b}	739.9±11.9^{c}

^{a,b,c} Means in the same line with different superscripts differ significantly ($P<0.05$).

3.2.3 Crude protein (CP)

The CP content of the four treatment diets and the CP contents of the feed refusals and faeces excreted by the young Dorper wethers in the four treatments are presented in Table 3.8.

As previously discussed (see 3.1.2), there were small differences in the CP content of the diets (Table 3.8). The small differences between the CP content of the diets and the relatively larger differences in the CP content of the feed refusals suggest that the wethers were selecting feed ingredients from the diets, which was reflected in the CP differences (Table 3.8) but not necessarily to the same extent in the DM (see 3.2.1; Table 3.6) or the OM (see 3.2.2; Table 3.7).

The CP content of the faeces (Table 3.8) did not differ significantly ($P>0.05$) between diets T0 and T12 and between diets T24 and T36, respectively, but the difference between diets T0 and T12 on the one hand and diets T24 and T36 on the other hand, were significant ($P>0.05$). This may be due to the fact that there was an increase in the amount of urea included in the diets commensurate with the incremental inclusion levels of *Opuntia* cladodes in the diets. Urea dissolves in the aqueous medium of the reticulo-rumen and the resulting NH_4^+ is utilized rapidly by the microorganisms in the reticulo-rumen or absorbed and recycled via the liver and saliva back to the reticulo-rumen (McDonald *et al.*, 2002); therefore, the differences in the CP content of the faeces may suggest that the utilization of CP derived from urea which requires no real digestion *per se* is higher than the other feed protein that has to be digested.

Table 3.8 The CP content of the diets, feed refusals and faeces excreted by the young Dorper wethers fed the four treatment diets with incremental levels of dried and coarsely ground *Opuntia* cladodes

	Treatment diets			
	T0	T12	T24	T36
Diet (g CP/kg DM)	167.9	180.9	165.4	168.0
Feed refused (g CP/kg DM)	126.9±7.1^{a}	134.7±17.9^{a}	144.6±13.5^{a}	142.1±17.8^{a}
Faeces excreted (g CP/kg DM)	164.2±4.1^{a}	156.6±4.5^{a}	140.9±10.0^{b}	139.3±4.5^{b}

^{a,b} Means in the same line with different superscripts differ significantly (P<0.05).

3.2.4 Ether extract (EE)

The EE content of the four treatment diets and the EE contents of the feed refusals and faeces excreted by the young Dorper wethers in the four treatments are presented in Table 3.9.

Table 3.9 The EE content of the diets, feed refusals and faeces excreted by the young Dorper wethers fed the four treatment diets with incremental levels of dried and coarsely ground *Opuntia* cladodes

	Treatment diets			
	T0	T12	T24	T36
Diet (g EE/kg DM)	23.5	24.9	25.8	27.0
Feed refused (g EE/kg DM)	11.3±5.2^{b}	17.9±4.5^{a,b}	22.2±5.4^{a}	15.5±4.1^{a,b}
Faeces excreted (g EE/kg DM)	26.1±5.0^{a}	26.7±4.7^{a}	22.8±5.0^{a}	24.2±7.1^{a}

^{a,b} Means in the same line with different superscripts differ significantly (P<0.05).

There were differences between the EE content of the diets and the feed refusals (Table 3.9), but these differences can not be explained readily. The EE content of the feed refusals differed significantly (P<0.05) between diets T0 and T24. The EE content of the feed refusals for diets T12 and T36 did not differ significantly ($P>0.05$) from each other or from treatment diets T0 and T24, respectively. No plausible reason can be offered for the higher EE content of the feed refusals of diet T24, but it might be related to diet selection for the component that contains most EE, which in these diets was the maize meal, a highly palatable feed; maize meal contains about 42 g EE/kg DM (McDonald *et al.*, 2002).

The EE content of the faeces of the wethers on the four diets did not differ significantly ($P>0.05$). The fact that the levels of EE in the faeces of wethers in some treatments are higher than the EE in their diets can possibly be explained by high levels of metabolic fats that were excreted (Schneider & Flatt, 1975).

3.2.5 Acid-detergent fibre (ADF)

The ADF content of the four treatment diets and the ADF contents of the feed refusals and faeces excreted by the young Dorper wethers in the four treatments are presented in Table 3.10.

Table 3.10 The ADF content of the diets, feed refusals and the faeces excreted by the young Dorper wethers fed the four treatment diets with incremental levels of dried and coarsely ground *Opuntia* cladodes

	Treatment diets			
	T0	T12	T24	T36
Diet (g ADF/kg DM)	304.0	265.6	239.2	207.2
Feed refused (g ADF/kg DM)	404.6±80.1^{a}	267.9±57.8^{b}	230.7±62.5^{b}	190.8±82.0^{b}
Faeces excreted (g ADF/kg DM)	402.9±18.3^{a}	376.6±6.2^{b}	361.7±10.2^{b}	308.4±12.2^{c}

^{a,b,c} Means in the same line with different superscripts differ significantly ($P<0.05$).

As previously discussed (see 3.1.1), there was a definite decrease in the ADF content of the four diets (Table 3.10) commensurate with the incremental inclusion levels of *Opuntia* cladodes. The feed refusals of diet T0 contained significantly more ($P<0.05$) ADF than those of the other treatments. Although there was a decrease in ADF content of the feed refusals, the differences between the three diets containing *Opuntia* cladodes (diets T12, T24 and T36) were not significant ($P>0.05$). These differences suggest that considerable diet selection for

ADF components occurred, similar to CP (see Table 3.8), although as discussed previously this was not reflected in the DM or the OM content of the food refusals.

The faecal ADF emanating from diets T0 and T36 were significantly different ($P<0.05$) from the other two diets. The faecal ADF content of diets T12 and T24 differed significantly ($P<0.05$) from diets T0 and T36, but not from each other ($P>0.05$).

3.2.6 Neutral-detergent fibre (NDF)

The NDF content of the four treatment diets and the NDF contents of the feed refusals and faeces excreted by the young Dorper wethers in the four treatments are presented in Table 3.11.

Table 3.11 The NDF content of the diets, feed refusals and faeces excreted by the young Dorper wethers fed the four treatment diets with incremental levels of dried and coarsely ground *Opuntia* cladodes

	Treatment diets			
	T0	T12	T24	T36
Diet (g NDF/kg DM)	470.8	410.2	410.0	315.0
Feed refused (g NDF/kg DM)	533.3±83.9^{a}	409.2±77.4^{a,b}	338.2±71.3^{b}	293.4±83.7^{b}
Faeces excreted (g NDF/kg DM)	544.9±19.4^{a}	503.7±18.7^{b}	475.8±6.2^{c}	420.0±14.5^{d}

^{a,b,c,d} Means in the same line with different superscripts differ significantly ($P<0.05$).

There appears to have been a decrease in NDF content of the four diets with inclusion of *Opuntia* cladodes (Table 3.11). The relatively big difference in NDF content between diets T0 and T36 is of particular interest. The NDF content of the feed refusals of diets T0 and T12 did not differ significantly ($P>0.05$) from each other. The NDF content of the feed refusals of the diets that contained *Opuntia* cladodes (diets T12, T24 and T36) did not differ significantly ($P>0.05$), but diets T24 and T36 differ significantly ($P<0.05$) from diet T0. These differences suggest that considerable diet selection for NDF components occurred similar to ADF and CP, although as discussed previously this was not reflected in the DM or the OM content of the food refusals.

The NDF content of the faeces emanating from the four diets differed significantly ($P<0.05$) from each other.

3.2.7 Gross energy (GE)

The GE content of the four treatment diets and the GE contents of the feed refusals and faeces excreted by the young Dorper wethers in the four treatments are presented in Table 3.12.

Table 3.12 The gross energy (GE) content of the four diets, feed refusals and faeces excreted by the young Dorper wethers fed the four treatment diets with incremental levels of dried and coarsely ground *Opuntia* cladodes

	Treatment diets			
Apparent digestibility coefficients	T0	T12	T24	T36
Diet (MJ GE/kg DM)	23.095	21.628	17.020	17.050
Feed refused (MJ GE/kg DM)	19.527±3.759^{a}	17.763±6.149^{a}	16.521±2.126^{a}	15.968±1.320^{a}
Faeces excreted (MJ GE/kg DM)	18.092±1.455^{a}	17.291±1.347^{a}	16.239±1.600^{a}	15.452±0.399^{a}

^{a,b} Means in the same line with different superscripts differ significantly ($P<0.05$).

The results in Table 3.12 show that the inclusion of the *Opuntia* cladodes caused a marked decrease in GE of the diets. This is likely due to the high mineral content of the *Opuntia* cladodes (Batista *et al.*, 2003), since minerals do not contribute to the energy value of a feed (McDonald *et al.*, 2002).

There were large but not significantly different ($P<0.05$) decreases in the GE of the refusals of diets T0 and T12. This suggests that the wethers were able to select for the higher energy containing ingredients in these diets, but the decrease in the GE of diets T24 and T36 was small indicating that less selection took place from these two diets.

The decrease in GE content differences between the diets and faeces was quite large for diets T0 and T12 and much smaller for diets T24 and T36. This suggests that the GE content of the diets containing higher inclusion levels of *Opuntia* cladodes (diets T24 and T36) was apparently not utilized as well as that of diets T0 and T12.

3.2.8 Apparent digestibility coefficients and digestible energy (DE)

The apparent digestibility coefficients for the different chemical constituents and the digestible energy (DE) content of the four treatment diets with incremental inclusion levels of *Opuntia* cladodes have been calculated (see 2.1.4.9) and are presented in Table 3.13.

The apparent digestibility coefficients of most chemical constituents increased with incremental inclusion levels of *Opuntia* cladodes in the diets (Table 3.13). There were no significant differences ($P<0.05$) in the apparent DM digestibility coefficients between diets T0, T12 and T24. Diet T36 differed significantly ($P<0.05$) from diets T0 and T12 but not from diet T24. Due to the low fibre content of *Opuntia* cladodes, which is positively correlated with low digestibility of a feed, it was expected that the *Opuntia* cladodes would be highly digestible (Nefzaoui & Ben Salem, 2000). It would then follow that the DM digestibility will increase with the higher inclusion levels of *Opuntia* cladodes in the diets.

The apparent OM digestibility coefficients of diets T0, T12 and T24 were not significantly different ($P>0.05$) (Table 3.13). Diet T36, however, differed significantly ($P<0.05$) from the other three diets. This was expected because the *Opuntia* cladodes contain higher levels of easily digestible carbohydrates (Ben Salem *et al.*, 1996; Tegegne, 2002).

The apparent CP digestibility coefficients of the diets increased significantly ($P<0.05$) with incremental inclusion levels of *Opuntia* cladodes (Table 3.12). This was probably due to the fact that urea was used to supplement the CP levels of the diets because the *Opuntia* cladodes had a much lower CP content (84.0 g CP/kg DM) than the lucerne hay (209 g CP/kg DM). Since urea is utilised very quickly and virtually completely by the microorganisms in the reticulo-rumen it was expected that the CP of diets T12, T24 and T36 would have reflected a higher apparent digestibility.

According to the results in Table 3.13 there was no significant difference ($P>0.05$) between diets in the apparent digestibility coefficients of the EE. This can be ascribed to the *Opuntia* not having a high EE content (see Table 3.1) and, therefore, it did not contribute to any real extent to the EE that needed to be digested.

Table 3.13 Apparent digestibility coefficients of the different chemical constituents and the digestible energy (DE) content of the four treatment diets with incremental levels of dried and coarsely ground *Opuntia* cladodes

	Treatment diets			
Apparent digestibility coefficients	T0	T12	T24	T36
DM	0.673 ± 0.005^{b}	0.674 ± 0.016^{b}	$0.689 \pm 0.024^{a,b}$	0.716 ± 0.022^{a}
OM	0.709 ± 0.006^{b}	0.711 ± 0.011^{b}	0.730 ± 0.019^{b}	0.759 ± 0.022^{a}
CP	0.687 ± 0.009^{c}	0.719 ± 0.015^{b}	$0.738 \pm 0.025^{a,b}$	0.768 ± 0.024^{a}
EE	0.651 ± 0.066^{a}	0.654 ± 0.063^{a}	0.730 ± 0.051^{a}	0.754 ± 0.078^{a}
ADF	0.554 ± 0.022^{a}	0.531 ± 0.024^{a}	0.531 ± 0.044^{a}	0.581 ± 0.030^{a}
NDF	0.627 ± 0.020^{a}	0.573 ± 0.023^{a}	0.611 ± 0.077^{a}	0.593 ± 0.064^{a}
DE (MJ/kg)	17.253 ± 0.476^{a}	15.898 ± 0.626^{b}	11.980 ± 0.403^{c}	12.689 ± 0.411^{c}

^{a,b,c} Means in the same line with different superscripts differ significantly ($P<0.05$).

The apparent ADF and NDF digestibility coefficients (Table 3.13) of the diets did not differ significantly ($P>0.05$) from each other. This suggests that the fibre present in the four diets was equally digestible. This was expected due to the fact that the *Opuntia* cladodes are low in fibre and thus the ADF that is present is mainly contributed by the lucerne and will therefore remain equally digestible. There lack of significant differences ($P>0.05$) (Table 3.13) between the apparent NDF digestibility coefficients of the diets can probably be attributed to the low NDF content of the *Opuntia* cladodes (see 3.1.1).

The DE of diets T0 and T12 differed significantly ($P>0.05$) from diets T24 and T36. The DE content of diets T24 and T36 (Table 3.13) did not differ statistically ($P>0.05$). The decrease in DE with incremental inclusion levels of *Opuntia* cladodes suggests that, even though the *Opuntia* cladodes contain more easily digestible carbohydrates (Ben Salem *et al.*, 1996; Tegegne, 2002), it still contains less DE than the lucerne hay that it replaced in these diets.

Thus, although the apparent digestible coefficients of the four treatment diets and the respective dietary constituents may not have varied much (Table 3.13), the DE intake of the wethers decreased with incremental inclusion levels of dried and coarsely ground *Opuntia* cladodes. This may have affected animal performance, an aspect that could not be evaluated in this trial. As was shown in Table 3.4, the voluntary DM intake of the wethers decreased with incremental inclusion levels of dried and coarsely ground *Opuntia* cladodes in the diets.

The longer term effects on animal performance needs further investigation.

4. Feed intake, digestibility and rumen fermentation trial

Cladodes of the spineless cactus pear *Opuntia ficus-indica* var. Algerian was used in this study and is referred to in an abbreviated format as *Opuntia* cladodes (see Chapters 2 and 3).

In this second trial of the study (see 2.1), four (4) rumen cannulated young Dorper wethers were used in a comprehensive evaluation of rumen variables in a crossover design (see Table 2.2). At the same time, the feed intake and digestibility of the four treatment diets with incremental inclusion levels of dried and coarsely ground *Opuntia* cladodes were also determined with the same rumen cannulated wethers.

The rumen variables are direct consequences of the feed ingested and the processes of rumen fermentation, therefore, the feed intake and digestibility of the four treatment diets determined in the second trial are presented first, followed by the rumen variables.

4.1 Feed intake and digestibility trials

After the first trial was concluded, fairly large quantities of the four treatment diets were still available for the second trial. However, it was estimated that the remainder of the four diets carried forward would not have been enough to conduct the second trial (rumen variables), therefore, a second batch of *Opuntia* cladodes was harvested on 3 Augustus 2004 (see 2.1.1 and 2.2.1).

The same procedures were used to harvest, process, dry and coarsely ground the *Opuntia* cladodes to pass through a 20 mm sieve in a hammer mill. Fresh batches of the four diets (T0, T12, T24 and T36) were then mixed as described previously (see 2.1.1); these freshly, mixed batches were added to and then thoroughly mixed with the remainder of the respective four diets carried forward from the first trial and used in the second trial of the study.

Due to an unfortunate mishap, plant material sampled from the second batch of *Opuntia* cladodes was not analysed separately as was done with the batch of *Opuntia* cladodes used in the first trial (see 3.1.2; Table 3.2).

4.1.1 Chemical composition of the four treatment diets

The chemical composition of the four diets (T0, T12, T24 and T36) with incremental inclusion levels of dried and coarsely ground *Opuntia* cladodes, is presented in Table 4.1

Table 4.1 Chemical composition of the four treatment diets with incremental inclusion levels of dried and coarsely ground *Opuntia* cladodes

	Treatment diets			
	T0	T12	T24	T36
DM (g DM/kg feed)	847.2±35.64^{a}	861.9±18.50^{a}	841.6±62.26^{a}	860.1±19.25^{a}
OM (g/kg DM)	905.0±1.9^{a}	903.7±7.1^{a}	888.1±3.6^{b}	886.8±4.6^{b}
CP (g/kg DM)	157.5±5.9^{a}	162.9±3.9^{a}	164.6±7.3^{a}	165.5±7.1^{a}
EE (g/kg DM)	24.2±2.4^{a}	22.9±2.4^{a}	23.7±1.9^{a}	23.3±1.6^{a}
ADF (g/kg DM)	268.7±14.6^{a}	228.7±13.1^{b}	207.5±12.9^{c}	189.8±4.5^{c}
NDF (g/kg DM)	476.7±26.9^{a}	417.4±47.2^{a,b}	376.6±30.3^{b,c}	344.7±34.2^{c}
GE (MJ/kg DM)	20.597±2.87	17.783±0.55	17.055±0.28	16.604±0.31

^{a,b,c} Means in the same line with different superscripts differ significantly ($P<0.05$).

The crossover design (see Chapter 2; 2.2.2; and Table 2.2) used in the second trial of the study, made it also possible to determine the feed intake and digestibility of the four diets consecutively during trial periods A, B, C and D with the four rumen cannulated Dorper wethers. Samples of the four diets (T0, T12, T24 and T36) offered to the wethers were collected, pooled and analysed for each of the four trial periods (A, B, C and D). Thus, it was possible to subject the chemical analysis of the four diets (Table 4.1) to a statistical analysis.

The DM content of the four diets (Table 4.1) did not decrease with increasing inclusion levels of *Opuntia* cladodes as was the case in the first trial (see Table 3.2). However, the DM content of the four diets was lower than those used in the first trial (Table 4.1 vs. Table 3.2). This can only be ascribed to the possibility that the DM content of the *Opuntia* cladodes of the second batch were lower than that used during the first trial.

The difference in OM content of diets T0 and T12 (Table 4.1) was not significant (P<0.05). Similarly, the OM content of diets T24 and T36 did not differ significantly (P<0.05) from each other. However, the OM contents of diets T0 and T12 were significantly (P<0.05) higher than for diets T24 and T36. This suggests that the *Opuntia* cladodes may have lowered the OM of the diets, mainly because of its high mineral content.

The CP content (Table 4.1) of the four diets did not differ significantly (P<0.05). The CP content of diets T0 and T12 were markedly less than found previously in the first trial (see Table 3.2), while the CP contents of diets T24 and T 36 were similar for the two trials. Therefore, the higher CP content of diet T12 discussed previously for the first trial (see 3.1.1) can only be ascribed to a sampling error.

The EE content of all four diets was low and did not differ significantly (P<0.05) between diets (Table 4.1). This supports a previous conclusion (see 3.1.2) that, despite the incremental inclusion levels of the dried and coarsely ground *Opuntia* cladodes, it did not contribute much to the total EE content of the four diets.

There was a significant (P<0.05) decrease in both the ADF and NDF contents of the four diets as the level of *Opuntia* cladode inclusion increased (Table 4.1). The *Opuntia* cladodes appear to have a greater influence on the NDF as reflected in the significant differences (P<0.05) between the four diets. The ADF content of diets T24 and T36 did not differ significantly (P<0.05) from each other. Though all values were markedly lower than in the previous trial (see 3.1.2), the tendency of the ADF to decrease with increasing inclusion levels of *Opuntia* cladodes is similar to what was previously found and discussed. The decrease in fibre content of the diets was expected because the lucerne hay supplied most of the fibre in the diets and the *Opuntia* cladodes, with its low fibre content, incrementally replaced some lucerne in diets T12, T24 and T36. The inclusion of the *Opuntia* cladodes appears to have a greater influence on the NDF as reflected in the significant (P<0.05) differences between the four diets. When the NDF content of the diets in this trial was compared with those from the previous trial (see Table 3.2), the NDF content of diets T0 and T12 were similar, while the NDF content of diet T24 was much lower than that of diet T36, which in turn was higher than previously reported. The difference in results cannot be explained readily, though the values are in line with the expected in the sense that the NDF content of diets generally decreases with the higher inclusion levels of *Opuntia* cladodes.

The GE content of the diets (Table 4.1) was markedly lower for diets T0 and T12 in this trial when compared with the previous trial; diets T24 and T36 had similar GE contents as reported previously (see 3.1.2). The tendency of the GE content to decrease with the higher inclusion level of the *Opuntia* cladodes remained the same as in the previous trial (see Table 3.2), though somewhat less pronounced.

4.1.2 Water intake and urine excretion

The *Opuntia* cladodes were dried and coarsely ground when it was included in three of the diets (T12, T24 and T36) to substitute coarsely ground lucerne hay. Similar to the first trial, the dried and coarsely ground *Opuntia* cladodes contributed much less water than in most trials where it was fed in the fresh or partially dried form with higher water content (Terblanche *et al.*, 1971; Ben Salem *et al.*, 1996). Again, similar to the results of the first trial, this may have affected both voluntary water consumption and urine excretion of the wethers in this trial, compared to the results reported in other studies (Terblanche *et al.*, 1971; Ben Salem *et al.*, 1996).

The daily water intake and urine excreted by the rumen cannulated young Dorper wethers fed the four treatment diets are presented in Table 4.2.

Table 4.2 Daily water intake and urine excreted by the rumen cannulated young Dorper wethers fed the four treatment diets with incremental inclusion levels of dried and coarsely ground *Opuntia* cladodes

	Treatment diets			
	T0	T12	T24	T36
Water intake (ml/day)	4972.1±1255.2^{a}	4850.0±669.0^{a}	5276.4±679.5^{a}	5165.0±1248.7^{a}
Urine excreted (ml/day)	1575.7±567.2^{a}	1360.7±252.4^{a}	1317.1±276.7^{a}	1392.1±230.6^{a}

^{a,b} Means in the same line with different superscripts differ significantly ($P<0.05$).

There was a dramatic increase in water intake and urine excreted by the wethers in this trial (Table 4.2) compared to the previous trial (Table 3.3). This is possibly due to the fact that the wethers were older and physically slightly larger, but more likely it may have been because

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this trial was conducted during spring (9 September to 28 October 2004) with relatively high ambient temperatures, in contrast to the previous trial that was conducted during the colder winter months. Commensurate with the sharp increases in water intake of the wethers, it also caused marked increases in the volume of urine excreted (Table 4.2).

In contrast with the results of the first trial (see 3.1.3), there were no significant ($P<0.05$) differences in the volume of water consumed by the wethers on the four diets. This suggests that the environmental temperature was the primary driving force in this trial for the higher water intake of the wethers during a period of higher ambient temperatures.

There was no significant ($P<0.05$) difference between the volume of urine excreted by the wethers on the four diets (Table 4.2). The volumes of urine excreted were markedly higher than the comparable urine excretions during the first trial (Table 3.3). The fact that the wethers on diet T0 excreted a slightly larger volume of urine in this trial, though not significantly different ($P<0.05$), supports the earlier conclusion that the dried and coarsely ground *Opuntia* mucilage containing cladodes might have retained more water while passing through the digestive tract of the sheep (see 3.1.3 and 3.1.4).

4.1.3 Feed intake and faeces excreted

The daily feed intake and faeces excreted by the rumen cannulated young Dorper wethers in the four treatments are summarised in Table 4.3.

Table 4.3 Daily feed intake and faeces excreted by the rumen cannulated young Dorper wethers fed the four treatment diets with incremental inclusion levels of dried and coarsely ground *Opuntia* cladodes

	Treatment diets			
	T0	T12	T24	T36
Feed intake (g DM/day)	1549.7 ± 256.2^{a}	1483.1 ± 172.0^{a}	1617.9 ± 288.5^{a}	1431.4 ± 337.9^{a}
Faeces excreted (g DM/day)	546.6 ± 116.1^{a}	$490.1 \pm 78.9^{a,b}$	536.3 ± 78.2^{a}	439.7 ± 118.9^{b}

^{a,b} Means in the same line with different superscripts differ significantly ($P<0.05$).

The feed intake and faeces excreted by the wethers in this trial are in agreement with the results and the conclusions made previously (see 3.1.4). There was no significant difference ($P<0.05$) in the levels of feed intake. There was however a significant ($P<0.05$) difference in the DM of faeces excreted by the wethers on some of the diets; diets T36 and T12 did not differ significantly ($P<0.05$) and diets T0, T12 and T24 were also not significantly different ($P<0.05$).

The daily feed intake per metabolic weight (kg $W^{0.75}$) of the wethers on the four treatment diets is presented in Table 4.4.

Table 4.4 Daily feed intake per metabolic weight (kg $W^{0.75}$) by the rumen cannulated young Dorper wethers fed the four treatment diets with incremental inclusion levels of dried and coarsely ground *Opuntia* cladodes

	Treatment diets			
	T0	T12	T24	T36
DM intake (g/kg $W^{0.75}$ per day)	86.7 ± 8.93^a	86.0 ± 8.88^a	93.3 ± 17.65^a	84.1 ± 14.43^a

^{a,b} Means in the same line with different superscripts differ significantly ($P<0.05$).

As mentioned previously (Table 4.3) there was no significant ($P<0.05$) difference in feed intake, but the DM intake per kg $W^{0.75}$ of the wethers was slightly higher for diet T24. The daily feed intake per metabolic weight (kg $W^{0.75}$) was higher (Table 4.4) compared to the results of the previous trial (see Table 3.4). This could be due to the fact that the wethers were more accustomed to the diets and the trial conditions because they had been exposed to it during the first trial. The wethers were also older and physically slightly larger than during the first trial, this would invariably have given rise to a higher feed intake. Furthermore, the higher feed intake could also be ascribed to the wethers being were maintained between the two trials on winter pasture (veld) and did, therefore, not have the same body condition than at the start of the previous trial, despite similar average body weights at the beginning of the two trials.

4.1.4 Body weight changes

The body weight changes of the rumen cannulated young Dorper wethers fed the four treatment diets are presented in Table 4.5.

There was no weight loss by the wethers during any time in this trial, even though the average body weight gain during the adaptation periods was much lower for diets T24 and T36 that contained the higher inclusion levels of *Opuntia* cladodes. There appeared to have been a substantial increase in body weight gain during the trial period, though the difference is not as great as during the first trial (see Table 3.5). The total body weight gain of the wethers in this trial was markedly different from the first trial. Diet T0 resulted in the largest increase in body weight (Table 4.5), while diet T36 promoted the lowest total body weight gain. In line with the results reported previously for the first trial, the body weight increases caused by diets T12 and T24 were again very similar.

Table 4.5 Body weight changes of the rumen cannulated young Dorper wethers fed the four treatment diets with incremental inclusion levels of dried and coarsely ground *Opuntia* cladodes

	Treatment diets			
Body weight changes (kg)	T0	T12	T24	T36
Four adaptation periods (7 days each)	2.550	1.575	0.275	0.625
Four trial periods (7 days each)	2.000	1.275	2.650	1.575
Total adaptation and trial periods (56 days)	18.200	11.400	11.700	8.800

4.2 Chemical composition of the diets, feed refusals and faeces

4.2.1 Dry matter (DM)

The DM content of the faeces excreted by the rumen cannulated young Dorper wethers in the four treatments is presented in Table 4.6.

Table 4.6 The DM content of the faeces excreted by the rumen cannulated young Dorper wethers fed the four treatment diets with incremental inclusion levels of dried and coarsely ground *Opuntia* cladodes

	Treatment diets			
	T0	T12	T24	T36
Faeces excreted (g DM/kg)	400.4±83.0^{a}	372.6±101.3^{a,b}	265.1±37.7^{b,c}	230.6±16.7^{c}

^{a,b,c} Means in the same line with different superscripts differ significantly ($P<0.05$).

There were no significant ($P<0.05$) differences in the water intake or the volume of urine excreted by the wethers on the four diets (see Table 4.2), therefore, it was not expected to find significant differences in the DM content of the faeces excreted by the wethers (Table 4.6). There was a marked decrease in the DM content of the faeces (Table 4.6) commensurate with the incremental inclusion levels of *Opuntia* cladodes in the diets. Some differences between diets were not significant ($P<0.05$), namely diets T0 and T12, diets T12 and T24, and diets T24 and T36, respectively. The DM content of the faeces excreted on diet T0 differed significantly ($P<0.05$) from diets T24 and T36 and those of diet T36 differed significantly ($P<0.05$) from diets T0 and T12. Furthermore, similar to the results of the first trial (Table 3.6), the faeces excreted on diet T36 was about twice as wet as those emanating from diet T0 (Table 4.6).

Again, similar to a conclusion drawn previously (see 3.2.1), it was not the inherent high water content of the *Opuntia* cladodes that caused the high water content of the faeces or conversely the low DM content, because the *Opuntia* cladodes were dried before inclusion into the diets. It is more plausible that this phenomenon of wet faeces is attributed to the action of the mucilage content and the great capacity of this hydrocolloid to imbibe water (Cárdenas *et al.*, 1997; Sáenz *et al.*, 2004); it could also have been attributed to the high oxalate content of cactus pears (Nefzaoui & Ben Salem, 2000).

4.2.2 Organic matter (OM)

The OM content of the diets, feed refusals and faeces excreted by the rumen cannulated young Dorper wethers in the four treatments is presented in Table 4.7.

Table 4.7 The OM content of the diets, feed refusals and faeces excreted by the rumen cannulated young Dorper wethers fed the four treatment diets with incremental inclusion levels of dried and coarsely ground *Opuntia* cladodes

	Treatment diets			
	T0	T12	T24	T36
Diet (g OM/kg DM)	905.0±1.9^{a}	903.7±7.1^{a}	888.1±3.6^{b}	886.8±4.6^{b}
Feed refused (g OM/kg DM)	884.2±9.9^{a}	859.4±15.0^{a}	854.6±19.2^{a}	860.8±8.0^{a}
Faeces excreted (g OM/kg DM)	879.7±5.6^{a}	825.9±26.9^{b}	806.4±25.2^{b}	785.4±12.6^{b}

^{a,b} Means in the same line with different superscripts differ significantly (P<0.05).

As reported previously (see 4.1.1), incremental inclusion levels of *Opuntia* cladodes decreased the OM content of the diets. There was however no significant difference (P<0.05) in the OM content of the feed refusals. This corresponds with the results found in the previous trial (see Table 3.7). The OM content of the feed refusals also corresponds with the OM contents of the feed refusals in the previous trial (see Table 3.7).

The OM content of the faeces (Table 4.7) excreted from the four diets was markedly higher than the OM content found in the previous trial (see Table 3.7). There was a significant (P<0.05) difference between the OM content of the faeces from diet T0 and the OM content of the other three diets. This was also found previously in the first trial, although then there was a significant (P<0.05) difference between diet T36 and the faeces from the other three diets.

4.2.3 Crude protein (CP)

The CP content of the diets, feed refusals and faeces excreted by the rumen cannulated young Dorper wethers in the four treatments is presented in Table 4.8.

Table 4.8 The CP content of the diets, feed refusals and by the rumen cannulated young Dorper wethers fed the four treatment diets with incremental inclusion levels of dried and coarsely ground *Opuntia* cladodes

	Treatment diets			
	T0	T12	T24	T36
Diet (g CP/kg DM)	157.5 ± 5.9^{a}	162.9 ± 3.9^{a}	164.6 ± 7.3^{a}	165.5 ± 7.1^{a}
Feed refused (g CP/kg DM)	145.6 ± 4.4^{a}	139.4 ± 13.9^{a}	138.2 ± 12.2^{a}	139.4 ± 28.1^{a}
Faeces excreted (g CP/kg DM)	158.7 ± 6.4^{a}	136.2 ± 33.1^{a}	147.3 ± 1.4^{a}	143.7 ± 3.1^{a}

^{a,b} Means in the same line with different superscripts differ significantly ($P<0.05$).

There was no significant ($P<0.05$) difference (Table 4.8) between the CP of the four diets (see 4.1.1) and there was no significant ($P<0.05$) difference between the feed refusals of the four diets. It can, however, be seen that there is again a difference between the CP in the diets and the CP in the feed refusals. The differences between the CP content of diets found in this trial is, however, not reflected in the feed refusals to the same extent that it was seen in the previous trial. The CP content of the feed refusals of diet T12 in the previous trial is similar to the CP content of the feed refusals found in this trial and not higher as would have been expected. It may again be assumed that the wethers were able to select the feed ingredients with higher CP content in the diets.

It this trial there was also no significant ($P<0.05$) difference between the CP of the faeces derived from the four diets. This result differs from that found previously in the first trial (see 3.2.2) where diets T0 and T12 were similar and differed significantly ($P<0.05$) from diets T24 and T36; the latter two diets in turn were similar.

4.2.4 Ether extract (EE)

The EE content of the diets, feed refusals and faeces excreted by the rumen cannulated young Dorper wethers in the four treatments is presented in Table 4.9.

When the results in Table 4.9 are compared to those found previously (see Table 3.9) only the feed refusals of diet T0 were different for the two trials. The EE contents of the feed refusals were higher than that in the previous trial. The EE contents of the feed refusals (Table 4.9) were not significantly ($P<0.05$) different between the four diets. This is contrary to the results of the previous trial where the feed refusals of diet T0 differed significantly ($P<0.05$) from diet T24 (see Table 3.9).

Table 4.9 The EE content of the diets, feed refusals and faeces excreted by the rumen cannulated young Dorper wethers fed the four treatment diets with incremental inclusion levels of dried and coarsely ground *Opuntia* cladodes

	Treatment diets			
	T0	T12	T24	T36
Diet (g EE/kg DM)	24.2±2.4^a	22.9±2.4^a	23.7±1.9^a	23.3±1.6^a
Feed refused (g EE/kg DM)	17.2±3.2^a	14.7±3.6^a	22.6±10.1^a	18.8±4.5^a
Faeces excreted (g EE/kg DM)	23.6±1.8^a	25.3±3.5^a	23.2±1.5^a	23.2±2.5^a

^{a,b} Means in the same line with different superscripts differ significantly ($P<0.05$).

The EE content of the faeces excreted by the wethers on the four diets in this trial was similar to that found in the previous trial (see Table 3.9) and there was also no significant ($P<0.05$) difference between any of the treatments.

4.2.5 Acid-detergent fibre (ADF)

The ADF content of the diets, feed refusals and faeces excreted by the rumen cannulated young Dorper wethers in the four treatments is presented in Table 4.10.

Table 4.10 The ADF content of the diets, feed refusals and faeces excreted by the rumen cannulated young Dorper wethers fed the four treatment diets with incremental inclusion levels of dried and coarsely ground *Opuntia* cladodes

	Treatment diets			
	T0	T12	T24	T36
Diet (g ADF/kg DM)	268.7±14.6^a	228.7±13.1^b	207.5±12.9^c	189.8±4.5^c
Feed refused (g ADF/kg DM)	333.6±52.0^a	293.1±89.8^{a,b}	209.0±64.8^{b,c}	157.8±37.0^c
Faeces excreted (g ADF/kg DM)	415.7±58.2^a	387.0±22.9^{a,b}	367.3±20.6^{a,b}	341.3±25.6^b

^{a,b,c} Means in the same line with different superscripts differ significantly ($P<0.05$).

As discussed previously (see 4.1.1), there were significant differences between the ADF levels of the four diets. The lower ADF content of the diets (see Table 4.1) is also reflected in the lower ADF content of the feed refusals compared with those of the previous trial (see Table 3.10) even though the differences were not significant ($P<0.05$). The only peculiar finding was a non-significant ($P<0.05$) increase in the ADF content of the feed refusals of diet T12. In the feed refusals (Table 4.10) there were no significant ($P<0.05$) differences between diets T0 and T12, diets T12 and T24, and diets T24 and T36, respectively. Previously the feed refusals of diet T0 (Table 3.10) differed from the other diets, while these latter three diets did not differ significantly ($P<0.05$) from each other.

An interesting finding in this trial was that the ADF contents of the faeces from all diets were slightly higher than in the first trial. With reference to the ADF content of the faeces from this trial there was a significant ($P<0.05$) difference between diets T0 and T36, while diets T0, T12 and T24 did not differ significantly ($P<0.05$) from each other and neither did diets T12, T24 and T36 differ. This result is in close agreement to what was found previously, although diets T0 and T36 differed significantly ($P<0.05$) from diets T12 and T24, while the latter did not differ significantly ($P<0.05$).

4.2.6 Neutral-detergent fibre (NDF)

The NDF content of the diets, feed refusals and faeces excreted by the rumen cannulated young Dorper wethers in the four treatments is presented in Table 4.11.

Table 4.11 The NDF content of the diets, feed refusals and faeces excreted by the rumen cannulated young Dorper wethers fed the four treatment diets with incremental inclusion levels of dried and coarsely ground *Opuntia* cladodes

	Treatment diets			
	T0	T12	T24	T36
Diet (g NDF/kg DM)	476.7 ± 26.9^{a}	417.4 ± 47.2^{b}	376.6 ± 30.3^{b}	344.7 ± 34.2^{c}
Feed refused (g NDF/kg DM)	516.9 ± 26.4^{a}	$454.5\pm82.5^{a,b}$	$372.9\pm73.6^{b,c}$	294.8 ± 78.5^{c}
Faeces excreted (g NDF/kg DM)	592.9 ± 30.7^{a}	512.2 ± 32.7^{b}	$491.9\pm29.1^{b,c}$	451.0 ± 19.6^{c}

^{a,b,c} Means in the same line with different superscripts differ significantly ($P<0.05$).

The low NDF content of the *Opuntia* cladodes had a significant ($P<0.05$) influence on the NDF content of the diets (see 4.1.1). The significant ($P<0.05$) differences in the NDF content of the feed refusals are also reflected in Table 4.11. A comparison between the NDF content and the feed refusals in the previous trial (see Table 3.11) showed that there was no significant ($P<0.05$) difference between the NDF content of the four diets. As with the ADF content in this trial (see 4.2.5), the feed refusals of diets T0, T24 and T36 had a slightly lower NDF content while the NDF content of the feed refusals of diet T12 was slightly higher. In this trial the NDF content of diets T0 and T12 differ significantly ($P<0.05$) from that of diet T36. The feed refusals of diet T0 is also significantly ($P<0.05$) different from diet T24. The feed refusals of diets T12 and T24 did not differ significantly ($P<0.05$) from each other and diet T24 also did not differ significantly ($P<0.05$) from diet T36. These results also reflect the general decrease in NDF content in the diets with incremental inclusion levels of *Opuntia* cladodes.

As was found with the ADF content of the faeces (see 4.2.5), there was a slight increase in the NDF content of the faeces of all four diets. The only change from the first trial was found in the case of diet T0, where the NDF in the faeces of the wethers differs significantly ($P<0.05$) from the other diets. The faeces emanating from diets T12 and T24 are similar and diets T24 and T36 do not differ significantly ($P<0.05$) either.

4.2.7 Apparent digestibility coefficients and digestible energy (DE)

The apparent digestibility coefficients of the different chemical constituents and DE of the four treatment diets are presented in Table 4.12.

The apparent DM digestibility coefficients of the diets (Table 4.12) were lower than in the first trial (see Table 3.13), but the increase in apparent DM digestibility coefficients of the diets with the higher inclusion levels of *Opuntia* cladodes was similar. In this trial the apparent DM digestibility coefficient of diet T36 was significantly ($P<0.05$) higher than that of diet T0. In this regard there were no significant ($P<0.05$) differences between diets T0, T12 and T24 or between diets T12, T24 and T36. This suggests that the inclusion of *Opuntia* cladodes had a slight effect of increasing the apparent DM digestibility of the diets.

Table 4.12 Apparent digestibility coefficients of the different chemical constituents and digestible energy (DE) content of the four treatment diets with incremental inclusion levels of dried and coarsely ground *Opuntia* cladodes

Apparent digestibility coefficients	Treatments			
	T0	T12	T24	T36
DM	0.649±0.022^{b}	0.671±0.019^{a,b}	0.667±0.017^{a,b}	0.695±0.017^{a}
OM	0.660±0.023^{b}	0.701±0.021^{a}	0.699±0.006^{a}	0.730±0.019^{a}
CP	0.648±0.043^{a}	0.726±0.080^{a}	0.707±0.008^{a}	0.740±0.017^{a}
EE	0.667±0.033^{a}	0.649±0.026^{a}	0.675±0.022^{a}	0.701±0.047^{a}
ADF	0.447±0.024^{a}	0.432±0.028^{a}	0.407±0.037^{a}	0.464±0.052^{a}
NDF	0.558±0.049^{a}	0.591±0.033^{a}	0.563±0.031^{a}	0.607±0.051^{a}
DE (MJ/kg)	13.2±2.5^{a}	11.7±0.4^{a}	11.0±0.8^{a}	11.6±0.7^{a}

^{a,b,c} Means in the same line with different superscripts differ significantly ($P<0.05$).

As with the other apparent digestibility coefficients of this trial compared to the previous trial, the apparent OM digestibility coefficients were also lower (see Table 3.13). In the previous trial it was more clearly seen that the incremental inclusion of *Opuntia* cladodes caused a gradual increase in the apparent OM digestibility coefficients of the diets. In this trial the higher inclusion level of *Opuntia* cladodes in diet T36 gave rise to a higher apparent OM digestibility, although those of diets T12 and T24 were also very similar. There was a significant ($P<0.05$) difference between diet T0 and the other three diets in terms of their apparent OM digestibility coefficients; however, diets T12, T24 and T36 did not differ significantly ($P<0.05$) from each other. Previously it was found that the apparent OM digestibility coefficients of diets T12 and T24 differed from diet T36 but not from diet T0.

The apparent CP digestibility coefficients of diets T0, T24 and T36 (Table 4.12) was lower than that of the previous trial (see Table 3.13) but it was slightly higher for diet T12. In contrast with results of the previous trial, where an increase in apparent CP digestibility occurred with the incremental inclusion levels of *Opuntia* cladodes, there was no significant difference ($P>0.05$) between the apparent CP digestibility coefficients of the four diets.

The apparent EE digestibility coefficients of the diets (Table 4.12) differed somewhat from the previous trial (see Table 3.13). In this trial, the apparent EE digestibility coefficient of diet T0 was slightly higher than in the previous trial, while those of diets T24 and T36 were much higher than previously reported. The apparent EE digestibility coefficient of diet T12 was slightly lower in this trial. Even with the different apparent EE digestibility coefficients of the two trials, there were in both instances no significant ($P>0.05$) differences between the four diets. This supports the conclusion that the inclusion of *Opuntia* cladodes did not affect the apparent digestibility of the EE in the four treatment diets to any real extent.

The apparent ADF digestibility coefficients of the four diets (see Table 4.12) were significantly lower than in the previous trial (see Table 3.13). As in the previous trial, the apparent ADF digestibility coefficient of diet T36 was the highest, while that of diet T0 was the second highest. Previously, the apparent ADF digestibility coefficients of diets T12 and T24 were very similar, but in this trial they differed slightly, but not significantly ($P>0.05$).

The apparent NDF digestibility coefficients of the four diets (Table 4.12) differed markedly from the results found in the previous trial (see Table 3.13). In this trial the apparent NDF digestibility coefficients of diets T0 and T24 were much lower than in the previous trial, while there was a slight increase in the values for diets T12 and T36. As previously reported, there was no significant difference ($P>0.05$) between the diets but the results of this trial reflects a slightly higher apparent NDF digestibility coefficient for diet T36, while in the previous trial, diet T0 had the highest apparent NDF digestibility coefficient.

The DE of diets T0 and T12 in this trial (see Table 4.12) were markedly lower than in the previous trial (see Table 3.13), while the DE for diets T24 and T36 were very similar. In this trial the DE of the diets were statistically similar ($P<0.05$), while in the previous trial there were significant differences ($P<0.05$) between most of the diets and decreasing as the *Opuntia* cladode content of the diets increased. In this trial, even though there was no significant ($P<0.05$) difference between diets, the DE of the three diets containing *Opuntia* cladodes (diets T12, T24 and T36) had a slightly lower DE than diet T0.

4.3 Rumen fermentation as reflected in rumen variables

The wethers were fed twice a day, namely at 08h00 and 16h00 (see 2.2.3.1). Therefore, based on the specific feeding regime used in this trial, the 24-hour cycle of evaluation could be subdivided into two periods: a first period of 8 hours, namely from 08h00 to 16h00 and then followed by a second period of 16 hours, extending from 16h00 to 08h00 the following morning.

4.3.1 Rumen pH

Utilization of potential energy sources by ruminants and the quantity and quality of amino acids available for absorption in the lower alimentary tract depends on the activity of the micro-organisms in the rumen (Chalupa, 1975; Ørskov, 1975; Satter & Roffler, 1975; Ørskov, 1977; Chalupa, 1984). Metabolic activity in the rumen usually reaches a peak within a few hours after feeding (Blackburn, 1965; Warner, 1965), with a concomitant decline in rumen pH (Du Plessis & Van der Merwe, 1970; Leng, 1970; Pritchard & Males, 1982). Diurnal variation in rumen pH is therefore an indication of the accumulation of organic acids in the rumen as a result of fermentation (Leng, 1970), as well as the secretion of saliva into the rumen (Church. 1973).

A rumen pH of between 6.2 and 7.0 is regarded as optimal for rumen activity (Ørskov & Ryle, 1990; Van Soest, 1994). The rumen pH of treatment T36 consistently fell within this range (Figure 4.1). The rumen pH of treatments T0, T12 and T24 were above 7.0 at 08h00 and 24 hours later at 08h00 the following morning. These results are similar to those of Ben Salem *et al.* (1996) who reported even higher rumen pH levels. There was however no significant difference ($P<0.05$) between the rumen pH of the diets at any specific time or on average over the 24-h cycle periods that the pH was measured.

The rumen pH of the four rumen cannulated wethers fed four treatment diets containing incremental levels of *Opuntia* cladodes are presented in Figure 4.1.

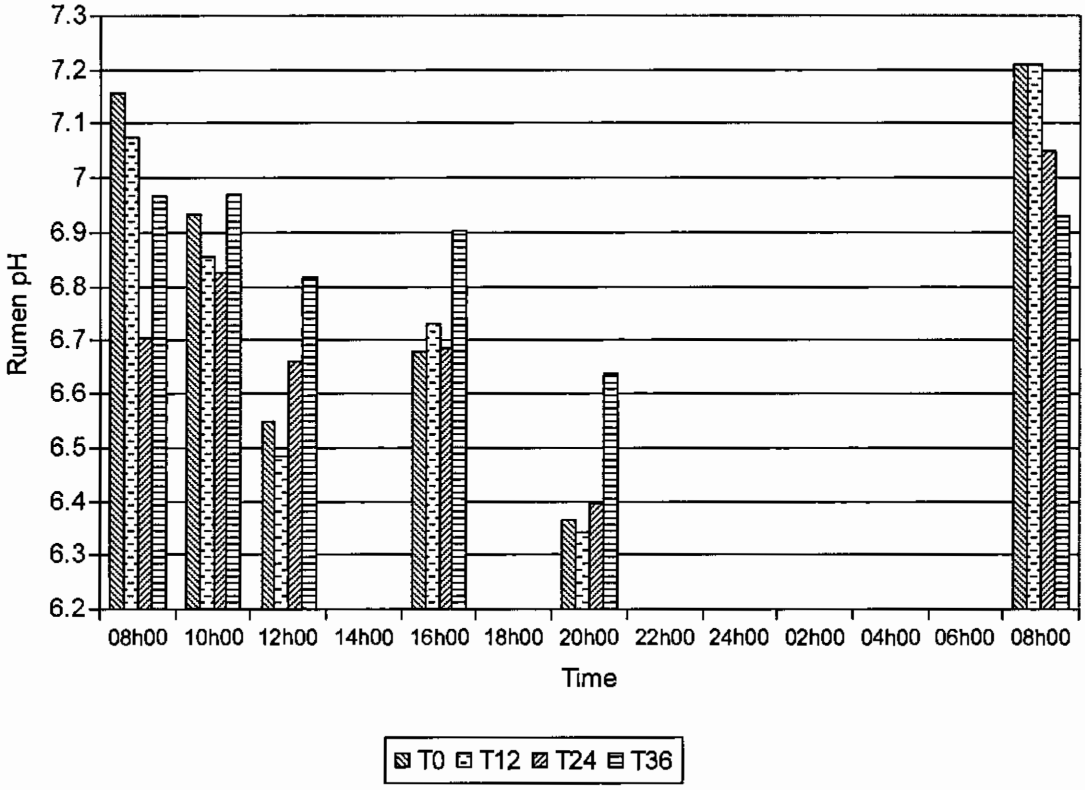

Figure 4.1 Diurnal variation of rumen pH relative to feeding at 08h00 and 16h00 of the rumen cannulated young Dorper wethers fed four treatment diets (T0, T12, T24 and T36) with incremental levels of dried and coarsely ground *Opuntia* cladodes.

When considering that cactus pear cladodes are highly digestible with high concentrations of rapidly digestible carbohydrates (Ben Salem *et al.*, 1996; Tegegne, 2002) it would be expected to find lower rumen pH levels, especially soon after feeding (De Waal & Biel, 1989). Ben Salem *et al.* (1996) attributes this phenomenon of a high rumen pH to the possibility that the consumption of large amounts of cactus pear probably enhanced salivation resulting from the high level of mineral salts and the abundance of mucilage in the cactus.

As would have been expected the rumen pH of all treatments declined over the first 4 hours post-feeding, thereafter (from 4 hours to 8 hours post-feeding at 08h00) there was a small rise in the rumen pH to 16h00 (Figure 4.1). The wethers were fed again at 16h00 in the afternoon and over the next 4 hours (from 16h00 to 20h00) there was a further decline in rumen pH; similar to what happened as a result of feed intake after 08h00 in the morning and the ensuing fermentation in the rumen (Du Plessis & Van der Merwe, 1970; Leng, 1970; Church, 1973; Pritchard & Males, 1982). The rumen pH of the wethers in all the treatments then probably decreased further before it rose again as reflected in the rumen pH measured at 08h00 the next morning (Figure 4.1).

4.3.2 Rumen ammonia (NH_3) concentration

Ammonia (NH_3) is utilized by most rumen bacteria as a primary N source (Bryant & Robinson, 1963; Hogan, 1975; Satter & Roffler, 1975; Chalupa, 1975; Schaefer *et al.*, 1980; Chalupa, 1984), but maximum rumen microbial protein synthesis depends on the availability of NH_3 in the presence of suitable fermentable substrates (Weston & Hogan, 1968; Hogan & Weston, 1970; Hogan, 1975; Satter & Roffler, 1975; Ørskov, 1977; Stern & Hoover, 1979; Chalupa, 1984). In this regard, 2-5 mg NH_3-N per 100 ml rumen fluid has been suggested as the optimum concentration for microbial protein synthesis (Satter & Slyter, 1974; Hogan, 1975; Satter & Roffler, 1975).

Rumen pH plays a major role in the absorption of both NH_3 (Bloomfield *et al.*, 1963; Blackburn, 1965; Leng & Nolan, 1984) and volatile fatty acids (VFA) from the rumen (Bloomfield *et al.*, 1963; Macleod *et al.*, 1984). Ionization of ammonia to ammonium (NH_4^+) is suppressed by a high rumen pH, thus more NH_4^+ is present at a lower pH. Ammonia is absorbed more rapidly in the un-ionized state (NH_3) (Bloomfield *et al.*, 1963; Blackburn, 1965; Leng & Nolan, 1984), therefore, NH_3-N is retained longer in the rumen at a lower rumen pH and, in the presence of suitable fermentable substrates, may result in higher microbial protein yields (Blackburn, 1965; Leng, 1984).

The rumen NH_3 concentrations of the four rumen cannulated wethers fed four treatment diets containing incremental levels of *Opuntia* cladodes are presented in Figure 4.2.

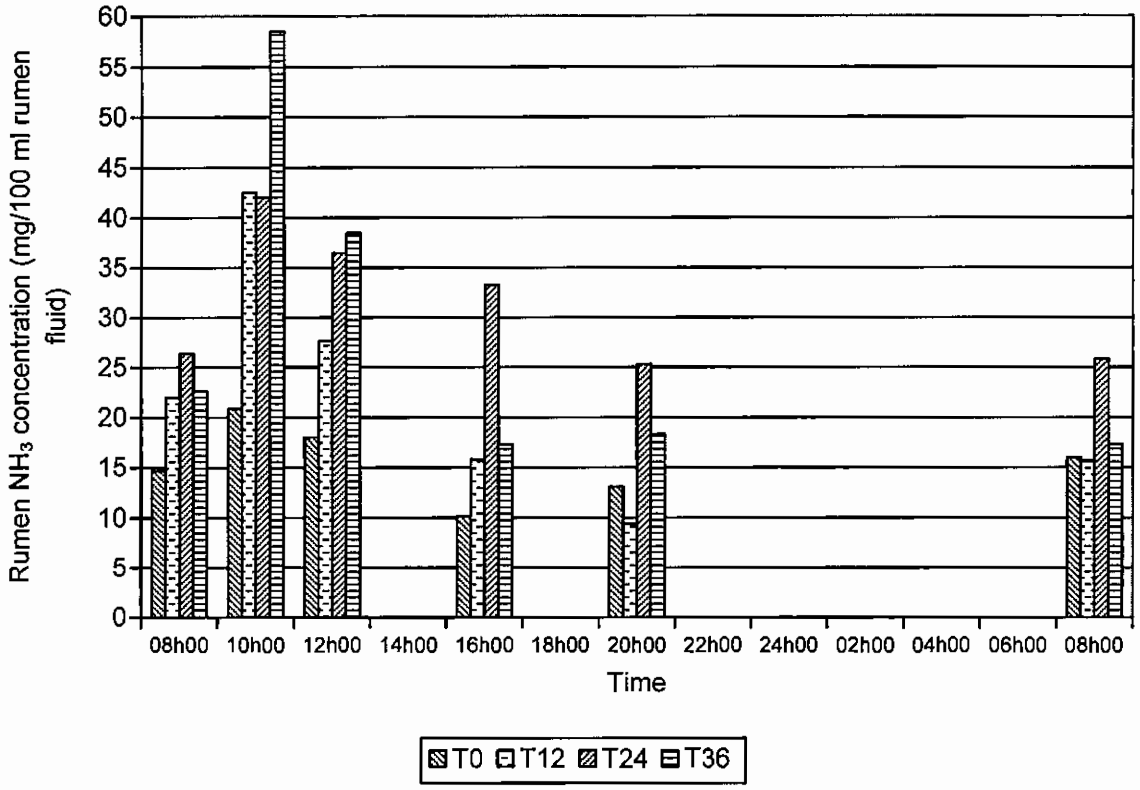

Figure 4.2 Diurnal variation of rumen NH_3 relative to feeding at 08h00 and 16h00 of the rumen cannulated young Dorper wethers fed four treatment diets (T0, T12, T24 and T36) with incremental levels of dried and coarsely ground *Opuntia* cladodes.

The rumen NH_3 concentrations peaked 2 hours post-feeding (10h00) and it was the only time that there was a significant difference ($P<0.05$) between treatments; treatments T0 and T36 differed significantly ($P<0.05$) from the other treatments. The peak in rumen NH_3 concentration of the treatment diets with the higher *Opuntia* cladode inclusion levels corresponds with results of Ben Salem *et al.* (1996). However, on average there was no significant ($P<0.05$) difference in the rumen NH_3 concentration between the various diets over the 24-h cycle of observation (Figure 4.2).

The concentration of rumen NH_3 was consistently high for all treatments (Figure 4.2). According to Van Soest (1994) the optimal ammonia level is 10 mg NH_3/100 ml rumen fluid, while Srinivas and Gupta (1997) suggested an optimal range of 2 to 30 mg NH_3/100 ml rumen fluid. Again, according to Van Soest (1994), the capacity of the bacteria for protein synthesis and NH_3 uptake depends on the rate of carbohydrate fermentation and that faster rates elicit greater efficiency and relatively higher NH_3 tolerance. The relatively high rumen NH_3 levels found in this trial (Figure 4.2) could be attributed to the high CP level of the lucerne (see 3.1.2; 209 g CP/kg DM) and also to the urea added as CP source to the three diets containing the *Opuntia* cladodes.

After the general sharp increase in rumen NH_3 concentration observed for treatments T12, T12 and T36 at 2h post-feeding (Figure 4.2; 10h00), the rumen NH_3 concentrations declined again over the next few hours. The sharp increase of the rumen NH_3 concentration of treatment T36 and to a lesser extent treatment T24 could be attributed to the effect of urea inclusion in the diets. Urea is highly soluble in water and would have been broken down rapidly by rumen microbial activity and converted to NH_3, resulting in the elevated rumen NH_3 concentrations.

Unlike the general response (decrease) to feeding at 16h00 seen for rumen pH (Figure 4.1), there was no similar response (increase) in rumen NH_3 concentration following feeding (Figure 4.2).

4.3.3 Rumen volatile fatty acid (VFA) concentration

According to Van Soest (1984) rumen VFA concentrations depend on the amount of VFA that are absorbed; it is the net result of rumen VFA produced and absorbed. The total rumen VFA concentrations rose steadily and reached a peak at 20h00 (12 and 4 hour post-feeding respectively as measured from feeding at 08h00 or 16h00) (Figure 4.3). This is in contrast to results of Ben Salem *et al.* (1996) who reported a peak occurring at 2 hours post-feeding. The peak in rumen VFA concentrations reached at 20h00 correspond with the lowest rumen pH measured (Figure 4.1) and is in agreement with reports by Van Soest (1984) and De Waal *et al.* (1989); both also showed a rise in rumen VFA concentrations commensurate with a drop in rumen pH.

The rumen VFA concentration of treatment T36 showed a peak at 4 hours post-feeding (12h00) after which there was a drop in rumen VFA concentration followed by another peak at 12 hours post-feeding (20h00). The rumen VFA concentration of treatment T12 also peaked at 4 hours post-feeding (12h00) and then remained more or less constant until 12 hours post-feeding (20h00). The general peak in rumen VFA concentration at 20h00 (12 or 4 hours post-feeding respectively) coincided with the drop in rumen NH_3 levels (Figure 4.2); this support an assumption that the high rumen NH_3 concentration possibly played a role in the observed rumen VFA concentration.

The rumen VFA concentrations of the four rumen cannulated wethers fed four treatment diets containing incremental levels of *Opuntia* cladodes are presented in Figure 4.3.

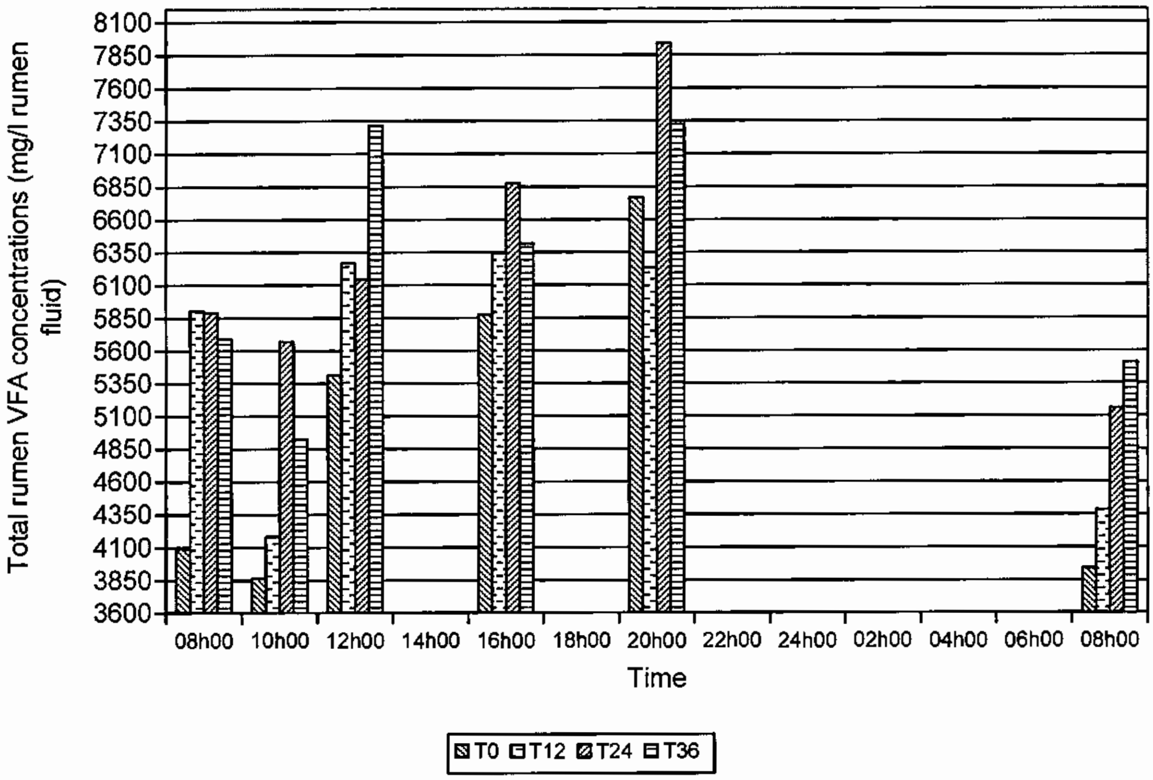

Figure 4.3 Diurnal variation of total VFA concentrations in the rumen relative to feeding at 08h00 and 16h00 of the rumen cannulated young Dorper wethers fed four treatment diets (T0, T12, T24 and T36) with incremental levels of dried and coarsely ground *Opuntia* cladodes.

4.3.3.1 Acetic acid concentration

The rumen acetic acid concentrations of the four rumen cannulated wethers fed four treatment diets containing incremental levels of *Opuntia* cladodes are presented in Figure 4.4.

At 2 hours post-feeding (10h00), the rumen acetic acid concentration of treatment T0 (Figure 4.4) differed significantly ($P<0.05$) from treatment T24. At 12h00 (4 hours post-feeding), the values of treatments T0 and T36 differed significantly ($P<0.05$) from each other. No other significant differences were observed during the 24 hour observation period.

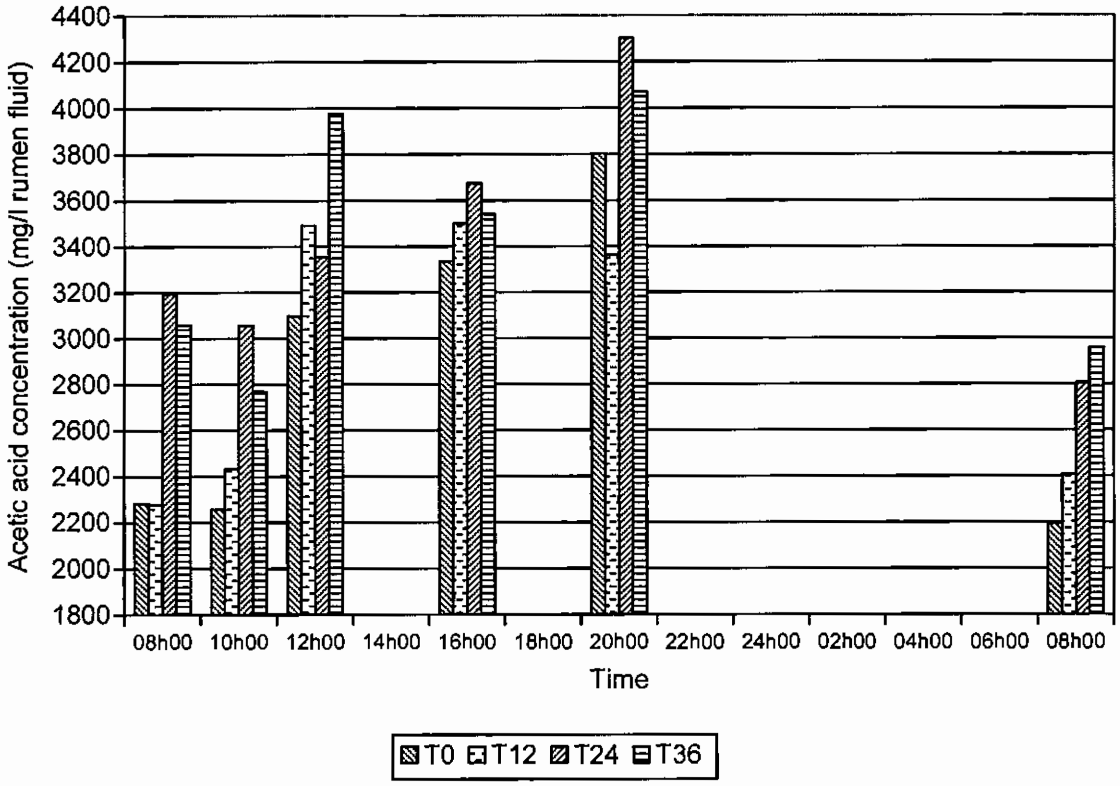

Figure 4.4 Diurnal variation of acetic acid concentrations in the rumen relative to feeding at 08h00 and 16h00 of the rumen cannulated young Dorper wethers fed four treatment diets (T0, T12, T24 and T36) with incremental levels of dried and coarsely ground *Opuntia* cladodes.

The acetic acid comprised between 50% and 60% of the total rumen VFA in all treatments. Treatment T12 at 08h00 (at 0 hours post-feeding) was an exception with the acetic acid comprising only 38% of the total rumen VFA. These values are slightly lower than the proportion of 0.65 derived from hexose reported by McDonald *et al.* (2002). This suggests that the inclusion of the *Opuntia* cladodes in the diets did not reduce the roughage fraction and causing a shift in microbial fermentation, thereby decreasing the rumen acetic acid concentration as could possibly be expected from a feed that contains highly digestible carbohydrates (McDonald *et al.*, 2002; López *et al.*, 2000; Ørskov & Ryle, 1990).

4.3.3.2 Propionic acid concentration

The rumen propionic acid concentrations of the four rumen cannulated wethers fed four treatment diets containing incremental levels of *Opuntia* cladodes are shown in Figure 4.5.

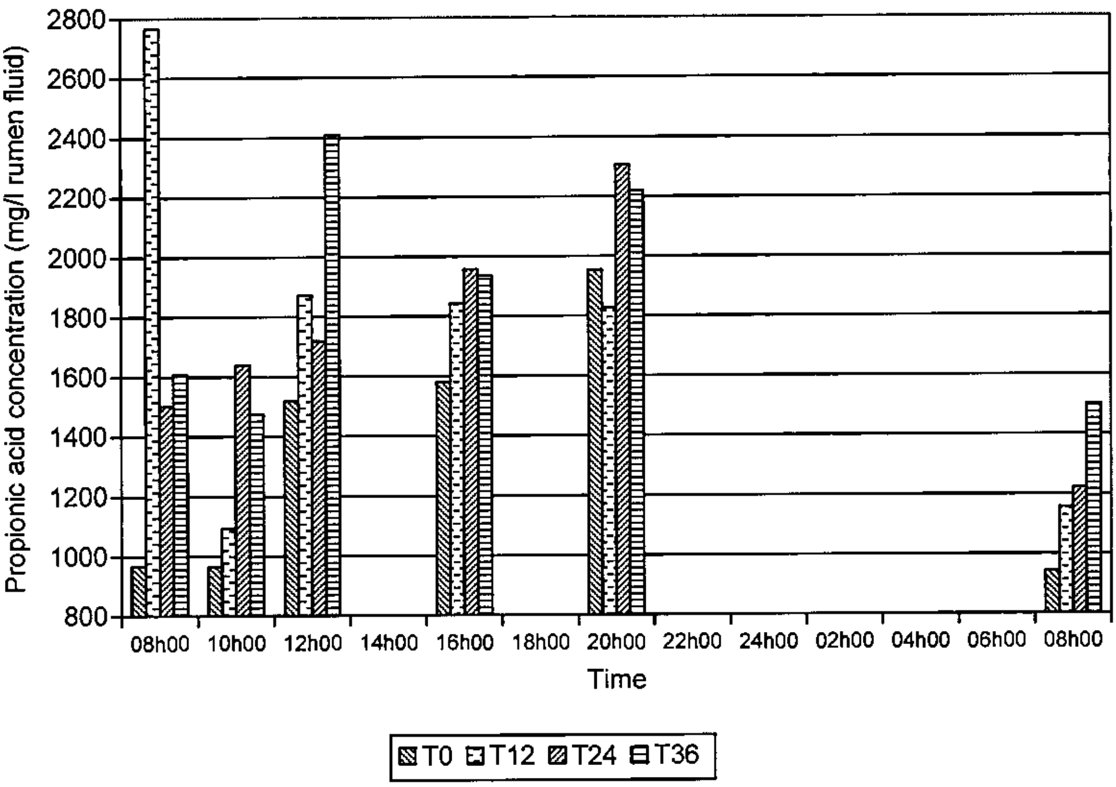

Figure 4.5 Diurnal variation of propionic acid concentrations in the rumen relative to feeding at 08h00 and 16h00 of the rumen cannulated young Dorper wethers fed four treatment diets (T0, T12, T24 and T36) with incremental levels of dried and coarsely ground *Opuntia* cladodes.

The only time post-feeding that there was a significant difference ($P<0.05$) in propionic acid concentration between diets was at 12h00 (4 hours post-feeding) where treatment T36 differed significantly from treatments T0 and T24 (Figure 4.5).

The proportion of propionic acid in the total pool of VFA ranged between 23% and 30%. At 12h00 the value for treatment T36 was slightly higher at 33% and the value for treatment T12 at 08h00 fell unexplainably far outside this range with a proportion of 47% (Figure 4.5). These values are slightly higher than the proportion of 0.21 for propionic acid derived from hexose (McDonald *et al.*, 2002). This suggests that while the inclusion of *Opuntia* cladodes in the diets did not affect the rumen fermentation, all the treatment diets contained fair amounts of easily fermentable carbohydrates leading to propionic acid production (McDonald *et al.*, 2002; López *et al.*, 2000; Ørskov & Ryle, 1990).

4.3.3.3 Butyric acid concentration

The rumen butyric acid concentrations of the four rumen cannulated wethers fed four treatment diets containing incremental levels of *Opuntia* cladodes are shown in Figure 4.6.

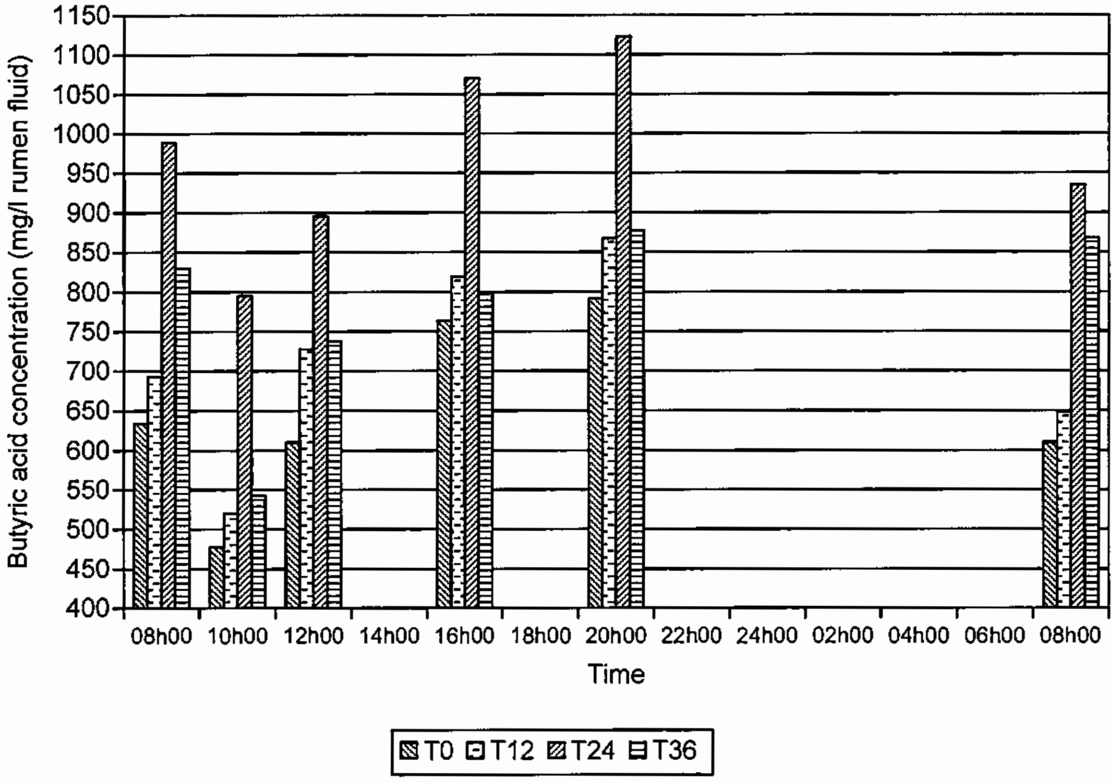

Figure 4.6 Diurnal variation of butyric acid concentrations in the rumen relative to feeding at 08h00 and 16h00 of the rumen cannulated young Dorper wethers fed four treatment diets (T0, T12, T24 and T36) with incremental levels of dried and coarsely ground *Opuntia* cladodes.

The results (Figure 4.6) showed that there was a significant difference ($P<0.05$) in butyric acid concentration between treatments T24 and T0 and T12 at 12h00 (4 hours post-feeding); this was the only time that there was a significant difference between treatments.

In the total rumen VFA pool the butyric acid proportion varied between 9% and 17%. According to McDonald *et al.* (2002) the proportion of butyric acid derived from hexose is 14%, suggesting that the values found in this trial fell within the normal range. According to McDonald *et al.* (2002) the proportion of butyric acid is less affected by diet than the other shorter chain fatty acids.

4.3.4 *In sacco* dry matter (DM) disappearance in the rumen

The *in sacco* DM disappearance in the rumen of the four rumen cannulated wethers fed four treatment diets containing incremental levels of *Opuntia* cladodes are shown in Figure 4.7.

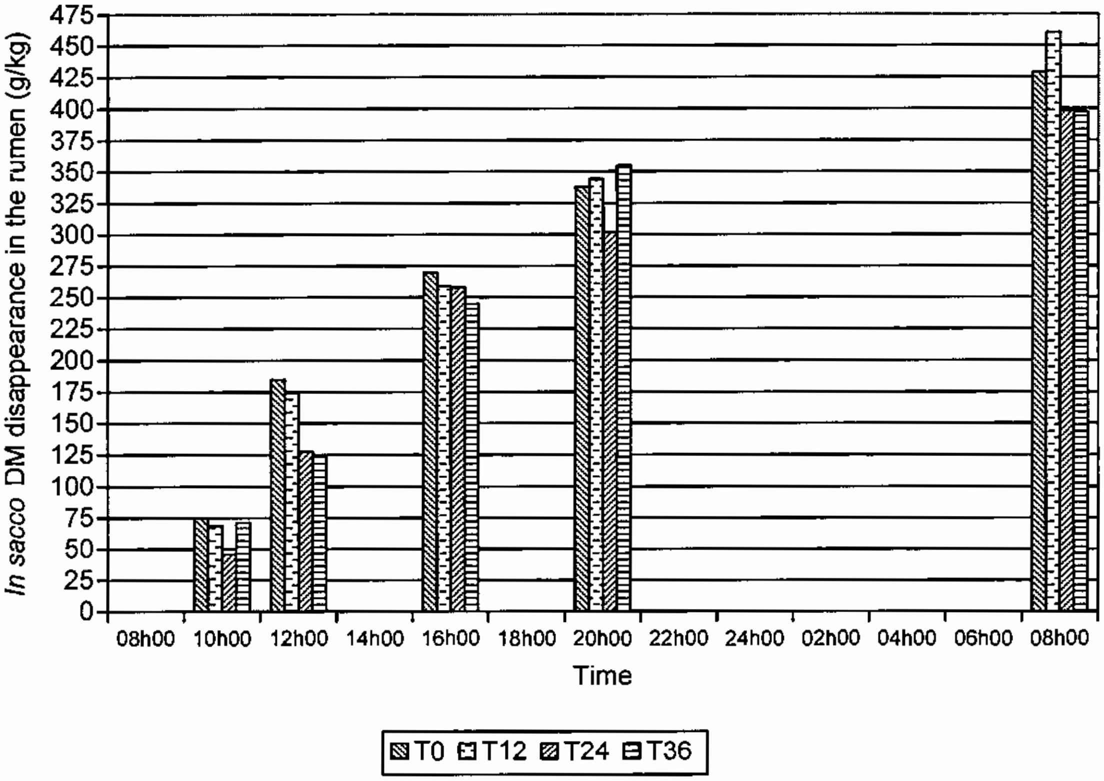

Figure 4.7 The *in sacco* DM disappearance in the rumen relative to feeding at 08h00 and 16h00 of the rumen cannulated young Dorper wethers fed four treatment diets (T0, T12, T24 and T36) with incremental levels of dried and coarsely ground *Opuntia* cladodes.

At 08h00, when the contents of the bags were only washed with water (see 2.2.4.1), there was no significant ($P<0.05$) difference between any of the treatments (Fig. 4.7). After 2 hour incubation in the rumen (at 10h00) treatment T36 differed significantly ($P<0.05$) from treatments T0 and T12. After 4, 8 and 12 hours incubation in the rumen, treatment T36 differed significantly ($P<0.05$) from treatment T0 but after 24 hours there was again no significant ($P<0.05$) difference between any of the diets.

4.3.5 General effects of rumen fermentation

It would seem that rumen fermentation was more affected by the feeding regime, namely diurnal events following feeding at 08h00 and 16h00 respectively, than what was observed as a direct result of the four treatment diets.

5. Conclusions and suggestions

The inclusion of dried and coarsely ground *Opuntia* cladodes to a level of 36% in balanced diets for sheep resulted in a decrease in the OM, CP, ADF, NDF and GE, but had no marked influence on the EE content of the diets. Addition of feed grade urea to three of the diets compensated for the lower CP of *Opuntia* cladodes and its incremental inclusion in these diets to substitute coarsely ground lucerne hay. The decrease in the OM, CP, ADF, NDF and GE of the diets as a result of the inclusion of the *Opuntia* cladodes seems to have been partially compensated for by the increases in the apparent digestibility of all these dietary components. The only exception was the decrease in DE at the higher inclusion levels of *Opuntia* cladode in the diets. In spite of the decrease in DE, the diets containing the higher levels of *Opuntia* cladodes yielded comparable results in terms of level of feed intake and animal production.

The daily voluntary water intake of the wethers increased significantly ($P<0.05$) with increasing inclusion levels of dried and coarsely ground *Opuntia* cladodes in the diets, but the differences in urine excretion were, however, not significant ($P>0.05$). This may be ascribed to the fact that the *Opuntia* cladodes contain a complex carbohydrate, mucilage, with a great capacity to absorb water. The precise function of the mucilage is not known, however, it is generally believed that it helps to retain water inside the cactus. Since mucilage binds strongly to water, it is quite plausible that this may render some water in the digestive tract of the wethers unavailable for absorption. Hence, the sheep may have needed to drink more water. The wethers on diet T36 (the highest inclusion level of *Opuntia* cladodes) drank on average about 900 ml water per day more than the wethers on diet T0, but they only excreted on average about 171 ml urine per day more than those on diet T0. Since the voluntary water intake of the wethers was significantly ($P<0.05$) higher on diet T36 (the highest inclusion level of *Opuntia* cladodes), it would have been expected that the water excreted in their faeces would also be markedly higher, which was not the case.

The feed intake of the young Dorper wethers decreased slightly with increasing levels of *Opuntia* cladodes in the diets, but these decreases were not significant ($P>0.05$). Similarly, by expressing feed intake per metabolic weight (g/kg $W^{0.75}$/day), the differences in feed intake were not significant ($P>0.05$). This suggests that incremental inclusion levels of the dried and coarsely ground *Opuntia* cladodes up to a level of 36% in the diet did not markedly change the acceptability or palatability of the diets for young Dorper wethers.

The DM excreted in the faeces by the wethers on the four treatment diets decreased slightly but the decreases were not significantly ($P>0.05$) different. This suggests that, since the DM intake of the wethers and the DM excreted in their faeces did not differ significantly ($P>0.05$) between treatments, the four diets were utilised with the same apparent efficiency.

Even when the *Opuntia* cladodes were dried in the sun before it was coarsely ground and included in the diets for sheep, the mucilage in the cladodes increased the water content of the faeces considerably, thus producing progressively very wetter faeces at the higher inclusion levels. The faeces were not typical of diarrhoea as often assumed when *Opuntia* is used in diets because it lacked the usual foul or offensive odours commensurate with diarrhoea. However, the very wet faeces of sheep may render the use of *Opuntia* cladodes in feedlot diets for sheep a less attractive proposition.

When considering the rumen variables that were studied in this trial the inclusion of dried and coarsely ground *Opuntia* cladodes had no significant ($P<0.05$) effect on the rumen pH, NH_3 and VFA concentrations as well as the rate of *in sacco* DM disappearance from the rumen. This suggests that the dried and coarsely ground *Opuntia* cladodes used in this study had no marked effect on rumen microbial activity and fermentation patterns in the rumen.

The use of dried and coarsely ground *Opuntia* cladodes in balanced diets for sheep as partial substitution for coarsely ground lucerne hay to an inclusion level of 36% is, therefore, a viable option to decrease the cost of sheep diets. In practical terms the greatest challenge to overcome will be the successful drying of large quantities of *Opuntia* cladodes, thus enabling farmers to transport it over longer distances from the production areas to where it can be used as livestock feed. This study offer some suggestions about cutting and drying of the *Opuntia* cladodes but further research is required to determine what possible influence the processing and drying of the cladodes in the sun may possibly has on its nutritional value.

More research is required on the role and especially the effects of mucilage on the digestive processes in ruminant feeds.

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