NUTRITIONAL EVALUATION AND PALATABILITY TRIAL OF ENSILED CONOCARPUS GREENERY RESIDUES

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SUMMARY

Conocarpus lancifolius is an ornamental tree planted in Kuwait, resulting in more than 120 t of residual by-products daily. These residues could be utilised as ensiled feed. A total premix of 24 t of silage was prepared in six pilot-scale trench silos for 30 days, after which triplicate samples were taken from each trench silo, which were analysed for nutritional contents and microbial populations. Mean pH value was 4.2 ± 0.12 , where mean of dry matter (DM), crude protein, crude fats, crude fibre, organic matter, lactic and acetic acids and total water soluble carbohydrate concentration were $35.10 \pm 4.0\%$, $11.18 \pm 0.07\%$, $2.47 \pm 0.21\%$, $20.85 \pm 0.95\%$, $19.53 \pm 0.53\%$, $4.95 \pm 0.32\%$, $1.47 \pm 0.01\%$ and $20.86 \pm 0.56\%$ on DM basis, respectively. Means of aerobic bacteria, fungi (yeast and moulds) and lactic acid bacteria counts were $1.8 \times 10^4 \pm 0.15$, $1.1 \times 10^4 \pm 0.09$ and $7.3 \times 10^8 \pm 0.12$ colony forming units per gram of fresh silage, respectively. An animal performance trial was conducted on 60 Holstein Friesian heifers with initial live weights ranging from 262 to 275 kg, grouped in six dietary treatments for a 148-day feeding trial. The diets contained different ratios of Conocarpus silage, which ranged from 10 to 60% on DM basis partially replacing conventional roughage (alfalfa hay, straw). DM intake of heifers ranged between 11.40 ± 2.14 kg and 13.61 \pm 2.54 kg. Daily live-weight gain ranged from 0.99 \pm 0.38 kg to 1.20 \pm 0.31 kg. From the results, Conocarpus silage could be suggested as a replacement for conventional roughage in rations for growing heifers.

INTRODUCTION

Animal feed shortage is the main constraint to the development of animal production in Kuwait. Over 90% of the total feeds required for local livestock in Kuwait are imported (Razzaque and Al-Nasser, 2003). Local livestock systems derive almost 40– 90% of feed requirements from forages. Recently, due to the soaring prices of livestock feeds the cost of livestock production has also increased. There is a great demand for home-produced good quality forage-based diets for livestock.

Conocarpus lancifolius, an ornamental tree of family *Combretaceae*, is native to coastal and riverine areas of East Africa. Because of its high salt and moderate drought tolerance, *C. lancifolius* is extensively used as an ornamental plant, as a pioneer abundant tree in reforestation projects and greenery programs and in landscaping in Kuwait and elsewhere in Arabian peninsula (Bhat *et al.*, 2009). In Kuwait, *Conocarpus* residues are found year-round with more than 120 t of by-products available on a daily basis. Although a small proportion of fresh greenery is fed to animals, most of the by-products

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are generally incinerated or dumped. *Conocarpus* is reported to be a non-toxic, palatable and attractive plant to feed animals because its greenery residual branches and shoots resulting from landscaping are used as fodder (Suleiman et al., 2005; ICBA, 2009). *Conocarpus* browse can be an inexpensive source of forage, and may be grazed, preserved or ensiled (Al-Surrayai and Baroon, 2005). Wensvoort (2008) studied the potential suitability of browse and produced green wastes from C. lancifolius in the United Arab Emirates (UAE) for silage to feed domestic and exotic animals. Owing to harsh and arid conditions and long summers in Kuwait, ensiling of wasted greenery by-products is considered as an alternative option, the popularity of which has increased over the past 10 years. As earlier studies have reported (Baroon et al., 2001, 2003, 2004, 2006), Conocarpus greenery residues are considered as an unconventional feed ingredient for ensiling. Ensilage of *Conocarpus* greenery residues was promoted as a pioneered step towards increasing feed availability from an environmentally sustainable source in Kuwait. Silage technology has opened the possibility of utilising green residues for livestock feeding (Marley et al., 2007; Willkinson, 2005). In many countries, silage accounts for more than 90% of conserved forages (Kaiser, 1993; Kaiser et al., 2004; Knickey, 2005).

The objective of the present study was to evaluate the nutritional value of *Conocarpus* silage as an upgraded ingredient by partially substituting conventional roughage in diets for growing Holstein Friesian heifers through animal performance trials.

MATERIALS AND METHODS

In order to determine the nutritional quality of silage with different ratios of added inoculants and molasses, experimental mini- and pilot-scale silage preparations in laboratory had been undertaken in studies conducted by Baroon *et al.* (2001, 2003, 2004, 2006). From the findings of these studies, *Conocarpus* silage described in this paper is considered as a good quality silage with applications of microbial inoculants at a rate of 2.0×10^9 colony forming units per kilogram (cfu kg⁻¹) and cane molasses at a rate of 6% as silage additives.

Conocarpus silage making

Fresh cuttings of *Conocarpus* residues were supplied by the House of Development (HOD) for Agricultural Contacting Company, a private entrepreneur for beautification, greenery and plants' maintenance. The silage prepared with 1–2-cm long browse, and consisting of residual green leaves and small branches, was shredded using a wood chipper (AB Alvan Blanch, UK). A total of six trench silos were filled with an average of 24-t plant material in each trench. A commercial microbial inoculum, 'KEMLAC®DRY' (Canada), was added as a microbial suspension at an application rate of 5.0 kg t⁻¹ with 2.0 × 10⁹ cfu kg⁻¹. The inoculum included a consortium of *Lactobacillus* species (*L. plantarum*, *L. caesi*, and *L. bulgaricus*) and enzymes (amylase and cellulase). Cane molasses was added at a rate of 6% during ensiling process by electric pasteurising sprayers. The total height of the silage was approximately 250 cm from the bottom of the trench silo. In order to minimise exposure to air before

covering, silage in different trenches were compacted in progressive layers using a wheeled Caterpillar tractor with a front-end loader. The trenches were filled to a height of 50 cm above ground surface and covered with thick plastic sheets. Finally, the surface of each trench silo was covered with a 0.5–1.0-m layer of soil, enabling continuous pressure and compaction. The ensiled material was left for 30 days.

Nutritional evaluation

After 30 days of anaerobic fermentation, representative samples of each trench in triplicate were taken for chemical and microbiological analysis.

A pH meter was used to measure pH values of fresh wet silage samples. For chemical analysis, samples were freeze-dried and grounded to pass through a 1–2-mm sieve. The DM content was determined by the removal of water using toluene distillation with ethanol correction as described by Dewar and MacDonald (1961). Crude protein, crude fat and organic matter contents were analysed according to the methods described by Association of Official Analytical Chemists (AOAC, 2002). Crude fibre was analysed according to AOAC (1995). Water-soluble carbohydrates (WSC) were determined by the spectrophotometric method described by Hall *et al.* (2000). Lactic, acetic, propionic and butyric acids were determined by high-performance liquid chromatography (HPLC) with ultraviolet visible detector as described by Ohmomo *et al.* (1993).

Microbial counts of fresh silage samples were determined using the plate count method according to methods described by AOAC (2002). Thirty gram of fresh silage was homogenised with 270 ml of sterile saline water (0.85% NaCl) by stomaching for 3 min with a laboratory blender (Seward Medical, London, UK). Serial dilutions of 10¹ to 10⁸ (ten-fold) were prepared. An aliquot of 0.1 ml of each dilution was spread over the surface of nutrient agar (NA Difco Laboratories, Michigan, USA) and potato dextrose agar (PDA Difco Laboratories, Michigan, USA) plates for enumeration of viable bacteria and fungi, respectively. Plates were incubated for 3 days at 30 °C. Populations of lactic acid bacteria (LAB) were counted on plate count agar (PCA Difco Laboratories, Michigan, USA) containing bromoresol purple. Plates were incubated in anaerobic jars at 35 °C for 3 days. Colonies were counted directly as colony forming units per gram of fresh silage.

Animal performance and diets

All the formulated dietary treatments comprised mixed concentrates and roughages in the ratio of 40:60 (Table 1). Control treatment, designated as T1, was a basal diet, which contained 40% of concentrate mixture and 60% of conventional roughages (alfalfa hay and straws) with no silage. Other dietary treatments – T2, T3, T4, T5 and T6 – also contained 40% of concentrates and 10, 20, 40, 50 and 60% of *Conocarpus* silage, respectively, thereby replacing partially the conventional roughage (alfalfa hay and straws). Basically, mixed concentrates in each dietary treatment, including the control treatment, comprised barley, corn, wheat bran, soya bean meal, vitamin and mineral supplements, limestone, salt and sodium bicarbonate at 14.6, 2.8, 10.0, 10.0,

Dietary treatment*	Alfalfa hay	Straw	Silage
T1	30	30	0
T2	25	25	10
T3	20	20	20
T4	10	10	40
T5	5	5	50
T6	0	0	60

Table 1. Formulated feeding rations for heifers % on dry matter (DM) basis.

*Dietary treatment = Tl (control) contained 60% conventional roughage (alfalfa hay and straws). T2, T3, T4 and T5 contained 10, 20, 40 and 50%, respectively, of *Conocarpus* silage partially substituted conventional DM of roughages. T6 contained 60% silage with no conventional roughage. All treatments contained 40% mixed concentrates of basal ingredients, i.e. 14.6, 2.8, 10.0, 10.0, 1.0, 1.0, 0.3 and 0.3% of barley, corn, wheat bran, soya bean meal, vitamin and mineral supplements, limestone, salt and sodium bicarbonate, respectively.

1.0, 1.0, 0.3 and 0.3%, of DM of the mix on DM basis, respectively. A total of 60 Holstein Friesian heifers, 10 months old with initial live weights ranging from 262 to 275 kg, were housed in a shed having pens measuring 10 m^2 and each fitted with a manger and water trough, for each group. Heifers were examined for any abnormality, diseases and initial breed characters. Prior to the trials, the animals were vaccinated for infectious diseases. Repeated two doses of anthelmintics were administered for control of internal and external parasites. Heifers were randomly grouped into six groups, with 10 heifers per treatment group. They were initially fed with basal diet of concentrate mixture and alfalfa hay ad libitum for a week. Then, Conocarpus silage was gradually introduced to animal feeds over a period of 3 weeks of adjustment to their diets and new housing. This period followed ad libitum feeding of experimental rations, and daily feed intakes were determined. The feeding trial consisted of a 28-day preliminary adaptation period, followed by a 120-day experimental period. The animals were fed twice a day, half of the feed was given in the morning and the other half in the evening. Feed ingredients were limited to basal concentrate of feed-grade barley, corn, wheat bran and soybean meal. Salt limestone, vitamins and mineral mixtures were also included. Wheat straw and alfalfa cubes and pellets were the basal roughages used for feeding. All the formulated diets comprised roughages and concentrates in the ratio of 40:60. Basically, major consideration was given to the replacement of conventional roughage components in the diets by the ensiled greenery Conocarpus residues. The diets were formulated to be isonitrogenous and isocaloric. The diet levels were determined during the adjustment period with specific emphasis on their nutritional needs such as DM, protein and energy according to the National Research Council (NCR) standards (2001). The leftover feeds, mixed diets with conventional roughages or silage, were collected in the following morning and the residual amounts were recorded. Refusals were quantified daily for adjustment of the next day's feed allocation, to allow ad libitum consumption of rations (10% refusals).

Nutritional parameter	$\mathrm{Mean}\pm\mathrm{SD}^*$
pH value	4.2 ± 0.12
Dry matter (DM)	35.10 ± 4.00
Crude protein	11.18 ± 0.07
Crude fats	2.47 ± 0.21
Crude fibre	20.85 ± 0.95
Organic matter	19.53 ± 0.53
Lactic acid	4.95 ± 0.32
Acetic acid	1.47 ± 0.01
Ratio of lactic acid to acetic acid	$3.38 \pm 0.11:1$
Total water soluble carbohydrates (WSC)	20.86 ± 0.56
Aerobic bacteria	$1.8 \times 10^4 \pm 0.15$
Fungi (yeasts and moulds)	$1.1 \times 10^4 \pm 0.089$
Lactic acid bacteria (LAB)	$7.3 \times 10^8 \pm 0.12$

Table 2. Mean values of pH, chemical composition (%) on dry matter (DM) basis and microbial enumerations (cfu/g) of fresh silage of *Conocarpus* silage.

*Standard deviation.

Dry matter intake consumed by the heifers was recorded. Live weight gain of heifers was taken once every month prior to the feeding time.

Statistical data analysis

Data on variations of nutritional composition of silage samples were evaluated by analysis of variance. The significance of differences among means was tested by the multiple range test (Duncan, 1955), and the significance was accepted at the p < 0.05 level. Feed intake and feed conversion efficiencies were subjected to the analysis of variance, assuming a randomised block design and treating the blocking group as a random effect as described by Payne *et al.* (2005).

RESULTS AND DISCUSSION

Nutritional quality

The mean values \pm standard deviation (SD) of nutritional parameters of the *Conocarpus* silage are given in Table 2. *Conocarpus* silage prepared with six replicates in six trenches was nearly similar in all the trenches. Hence, means of fermentation and nutritional parameters were calculated. After 30 days of ensilage, *Conocarpus* silage had an acceptable physical texture and odour, indicating good anaerobic fermentation. Odour is one of the best organoleptic parameters, indicating the fermentative quality of silage (Muck *et al.*, 2003; Ruppel *et al.*, 1995). The silage had acceptable pH with mean pH \pm SD of 4.2 \pm 0.12 (Table 2). *Conocarpus* silage's pH is the best single indicator of the effect of ensiling on the nutritive value of silage. In general, lower pH is better because it indicates that a lactic acid type of fermentation has taken place. Under anaerobic conditions, low pH of silage inhibits the growth of undesirable micro-organisms such as clostridia (Woolford and Pahlow, 1998). High pH indicates

poor forage fermentation, characterised by high levels of butyric acid, (McDonald et al., 1991). Contents of crude protein, crude fats and fibre and organic matter of Conocarpus silage were calculated as percentage on DM basis (Table 2). In general, silage is considered palatable with a protein content ranging from 7.0 to 11.0% on DM basis (Shehata et al., 2004). As reported by Selmer-Olsen et al. (2006), the lactic acid of silage partially hydrolyses the crude fibre and synergistically enhances the enzymatic activity of cellulase through slow chemical hydrolysis. The ratio of lactic acid to acetic acid is a good indicator of the efficiency of the silage fermentation. As the ratios are higher than the recommended value of 3:1, the quality of the prepared *Conocarpus* silage was suggested to be excellent. Concentrations of total WSC, lactic acid and acetic acid were within the standard limits (Table 2). Haigh (2006) ideally found that the critical WSC concentration for successful preservation was 30 g kg^{-1} DM where LAB utilise fermentable carbohydrates, such as fructans, and hexose sugars, i.e. glucose, galactose, mannose, xylose and arabinose, during ensiling. Negligibly low concentration of propionic acid, not exceeding 1%, was detected in the prepared silage, while butyric acid was undetectable (not shown in the table). In an ideal fermentation, lactic acid ranges from 4 to 6% on DM basis; acetic acid is usually found between 1 and 3%, while propionic and butyric acid contents should be undetectable (Haigh, 2006). High lactic acid, ratio of lactic acid to acetic acid, moderate acetic acid concentration combined with undetected butyric acid concentration are indicative of good anaerobic fermentation. Lactic acid should make up to at least 70% of the total acids in a good silage, whereas elevated concentration of acetic acid may result in continued fungal growth, excessive heating and subsequent loss of available protein, DM and energy. High butyric acid contents in the silage may result in clostridial fermentation and may have a negative impact on animal health and performance.

Microbiological determination

The results of aerobic bacteria, fungi (yeasts and moulds) and LAB of *Conocarpus* silage are shown in Table 2. Aerobic bacteria, fungi and LAB populations differed significantly ($p \le 0.05$) in the silage. Inoculation of the silage with microbial inoculants resulted in an increased count of LAB, i.e. up to 10^8 cfu g⁻¹ of silage after 30 days of fermentation (Table 2). The addition of LAB inoculant tended to ensure vigorous fermentation and rapid accumulation of lactic acid. Considering our data, population size of LAB (10^8 cfu g⁻¹ silage) was an indication of excellent ensiling process. Bolsen *et al.* (1995) and Kung and Ranjit (2001) considered *L. acidophilus, L. plantarum* and *Streptococcus durans* as potential microbial inoculants in silage making process according to their ability in rapid acidification and low pH value. This has been strongly proved by the research conducted by Nkosi *et al.* (2009). Research in laboratory-scale studies carried out by Bolsen *et al.* (1992) and Bolsen (1997) showed that inoculated silage had faster and more efficient fermentations, lower pH, higher lactic content, higher lactic acid to acetic acid ratio and lower ethanol and ammonia concentrations.

According to nutritional analysis, the overall evaluation of the prepared *Conocarpus* silage indicated efficient anaerobic fermentation with low pH values. Acceptable

Dietary treatments*	DM intake (kg) \pm SD [†]	Weight gain (kg) \pm SD
T1	10.00 ± 0.58	1.07 ± 0.26
Т2	11.23 ± 1.43	1.20 ± 0.35
T3	11.4 ± 2.14	1.12 ± 0.31
T4	13.09 ± 2.79	1.11 ± 0.34
T5	13.61 ± 2.54	1.15 ± 0.45
Т6	13.20 ± 2.17	0.99 ± 0.38

Table 3. Daily dry matter (DM) intake, and live weight gain of dairy heifers.

*Refer to Table 1 footnote for description of dietary treatments.

[†]Standard deviation.

contents of DM, crude protein, crude fats and crude fibre were detected. Relevantly, high lactic acid and low acetic acid concentrations, high lactic acid to acetic acid ratios, insignificant concentrations of propionic acid and undetectable concentrations of butyric acid were determined. In addition, high LAB counts, low viable bacterial and yeast counts were also recorded.

Performance of heifers

The general health of the animals remained good throughout the 148-day experimental period. Heifers on treatments grew satisfactorily, thereby indicating that health problems were insignificant.

DM intake and live weight gain of heifers

Means of DM intake and live weight gains of heifers during the entire 148-day trial are shown in Table 3. The experimental design had limitations because each pen of animals was considered a treatment group. In other words, the statistical evaluation of feed consumption was limited due to insufficient replication of pens with uniformly weighed heifers. Nevertheless, comparative animal performance parameters were clearly observed in this trial. After the adjustment period of 28 days, the heifers were accustomed to the silage-containing ration. The DM intake by T2 and T3 heifers were 11.23 \pm 1.43 kg and 11.4 \pm 2.14 kg, respectively. The difference in DM intake by T2 and T3 heifers was not significant when compared with T1 heifers (10.00 \pm 0.58 kg; Table 3). Remarkably, DM intake was significantly high, i.e. 13.09 \pm 2.79 kg, 13.61 \pm 2.54 kg and 13.20 \pm 2.17 kg in T4, T5 and T6 that contained 40, 50 and 60% silage, respectively, when compared with T1 ($p \leq 0.05$).

Mean values of live weight gains of the growing heifers during the 148-day trial are shown in Table 3. All the heifers grew throughout the study period. However, the overall differences in live weight gains of heifers were not significant (p > 0.05) among the six dietary treatments even after the adjustment period.

In this trial, DM intake with high levels of concentrates (40%) in the diets was acceptable. The complete rich diet containing concentrates combined with good *Conocarpus* silage lead to high growth rates for the experimental heifers groups. Barrière *et al.* (2004), Chizzotti *et al.* (2009), Dalton *et al.* (2008) and Rodrigues *et al.* (2002),

reported satisfactory DM intake with appropriate concentrate levels of diets containing grass hay or silage as roughage. On the other hand, Carvalho *et al.* (2006) and Evans *et al.* (2004) found no effect of concentrate ratios on DM intake, while Pereira *et al.* (2008) traced digestibility parameters in beef cattle-fed diets containing silage and concentrates. Kaiser (1993) conducted field trials, which showed that high-quality silage could be successfully used in finishing diets for cattle to support satisfactory animal performance, produce high-quality carcass and meat suitable for the domestic market and significantly improve net returns per head.

CONCLUSIONS

This study was an initial approach to utilising *Conocarpus* silage as a feed ingredient for ruminants. *Conocarpus* silage-containing rations were palatable, and based on the findings they are suggested as alternative roughage to the combinations of alfalfa hay and straw in the diets of growing heifers.

An overall nutritional evaluation of *Conocarpus* silages would address the potential for large-scale silage making. Further research is required to broadly define the nutritional quality of *Conocarpus* silage in farm-scale silos through comprehensive animal performance trials.

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