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Research Article

COMPARISON OF *IN VITRO* DIGESTIBILITY OF DIETS CONTAINING SUBABUL PLANT AS FODDER IN DROMEDARY CAMEL AND COW

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ABSTRACT: The aim of this study was to compare *in vitro* digestibility of diet containing Subabul (*Leucaena leucocephala*) at blooming stage in cow and dromedary camel. The experimental diets were including 0, 25, 50 and 100% of Subabul branches as a replacement of Alfalfa (*Medicago sativa*). The ingredients of diets were Alfalfa, wheat straw, corn silage, corn, barely, bran and mineral-vitamin supplement by a ratio of concentrate and forage at 50:50. Rumen fluid was taken from fistulated cow and dromedary camels and *in vitro* digestibility of experimental diets was determined. The obtained data were analyzed in a split plot design. The lowest digestibility of dry matter in dromedary camel (73.1%) and cattle (71.9%) was found in the treatment containing 100% of Subabul ($p<0.05$). But digestibility of natural detergent fiber (NDF) and acid detergent fiber (ADF) of diets containing different levels of Subabul instead of Alfalfa were not different in cow and camel ($p>0.05$). Digestibility of NDF and ADF in the treatment containing 100% Subabul in the cattle were 70.36 and 68.21%, respectively, and in the dromedary camel were 71.19% and 69.02%, respectively ($p<0.05$). Treatments containing 50 and 100 % Subabul (74.38 and 70.37%) had the lowest dry matter digestibility ($p<0.05$). The ADF digestibility in treatments containing 50 and 100% Subabul in compared with control treatment was 72.4 and 67.6 vs 73.5 %, respectively, and NDF digestibility was 72.45, 69.65 and 75.33 % in treatments containing 50 and 100% Subabul and control, respectively ($p<0.05$). As per the result, the digestibility of DM, NDF and ADF of Subabul were not different between cow and dromedary camel ($p>0.05$).The result of these experiments showed that *in vitro* digestibility of 50 % Subabul instead of Alfalfa in diet of cow and camel was better than other levels, therefore it can be used in cow and dromedary camel nutrition.

Key words: *In vitro* digestibility, Subabul, Non-conventional feed source, Dromedary camel, Cow.

INTRODUCTION

The competition on the natural resources for feed soughs have motivated nutritionists to use nonconventional fodder sources as part of the animal feed. The multi-purpose foliage (as a cheap source of nitrogen energy, minerals and vitamins) has the potential to be used in fulfilment of the nutrient requirement of the ruminants (Patra *et al*. 2010). The fodder trees (multipurpose foliage) have the tremendous potential in adaptation of the challenges of its ecosystem, ensuring abundant and continuous supply of feed stuffs. But the major problem of these uncommon resources and trees leaves is the presence of anti-nutritional agents such as tannin and its negative effects on livestock.

Subabul (*Leucaena leucocephala*) belong to leguminous family and is nonconventional feed resource for ruminants in most tropical and subtropical areas (Rodríguez *et al*. 2014, Meena devi *et al*. 2013). Subabul grows in warm and humid area of North, Central and South America and South Mexico, India, China, Japan, Malaysia, Indonesia, the Philippines, Australia and South Africa (Mozafaryan 2008). This tree introduced in Khuzestan province, Bushehr and Hormozgan in Iran.

The Subabul is an evergreen tree with a longer life span and rapid growth with a height of about 7 meters (Mozafaryan 2008). Growing of the Subabul is possible by seeding early in autumn (Poonam and Push 1994). Subabul is resistant to grazing and drought and its leaves

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are rich in beta-carotene and calcium and phosphorus. Leaves and branches produce more than 3 tons per hectare of crude protein that amino acids are same with alfalfa. Because of its high protein and low fiber of Subabul, it can be used as a good supplement for ruminants (Poonam and Push, 1994). Researchers reported (Nasimi *et al.* 2016) that crude protein of Subabul leaves, pods and stems is 14% to 30 %. Also, NDF, ADF, and fat of Subabul leaves are reported as 40.9%, 25.4% and 4%, respectively. The amount of dry matter, crude protein, crude fiber, ash, total soluble carbohydrates and lignin of Subabul pods were 91.4%, 16%, 35.6%, 4.9%, 27.4% and 11.74% dry matter (Shahriari *et al.* 2017).

Subabul contains mimosin, tannin, saponin, oxalate and alkaloids (Dryden 2008). Mimosin is a non-protein amino acid found in all parts of the Subabul in variable proportions. It has generally been accepted that Subabul can form 30% of the non-adapted diet in ruminants without any toxic effects (Gupta and Atreja 1999). Researchers (Islam 1995) reported a total amount of tannin in Subabul pods and leaves is 2.4% and 3.2%, respectively, which may cause poisoning in the animal (Eroaram 2002).

Subabul forage improves the digestibility of dry matter and protein in low-quality diets (Soltan *et al.* 2012). Also, daily feed intake, the ammonia production and volatile fatty acids in the rumen increased (Islam 1995). The camel has many unique qualities to survive and serve under harsh climate and unpleasant situations of wasteland and semi-wasteland regions. They also utilize low quality feed resources which other species cannot consume (Jouany *et al.* 1992).

Therefore, the purpose of this experiment was to investigate the effect of replacing alfalfa with Subabul fodder on *in vitro* digestibility, in cattle and dromedary camel.

MATERIALS AND METHODS

Experimental diets and chemical analyses

The Subabul branches (mixture of leaves, pods and flower) were collected and chopped. In this experiment, the alfalfa was replaced by Subabul fodder in proportions of 0 (control), 25%, 50% and 100%. The diets formulated according to NRC (2001) are shown in Table 1. The content of dry matter (DM), ash, crude protein (CP), neutral detergent fiber (NDF), ether extract (EE) and total tannin (TT) of mixture of Subabul leaves+pods+flowers were 918, 80, 244, 341, 98 and 38 g kg^{-1} of DM, respectively.

Subabul samples were oven-dried at 55 ^{o}C for 48 h and then were passed through a 1 mm sieve (Wiley mill, Swedesboro, USA). DM, ash, CP and EE were analyzed according to the methods of AOAC (1999). Then, NDF and ADF were analyzed using the method of Van Soest *et al.* (1991). Acid detergent lignin (ADL) was determined by solubilization of cellulose with a sulfuric acid (Van Soest *et al.* 1991).

In vitro digestibility

The *in vitro* digestibility of dry matter, NDF and ADF of experimental diets containing Subabul fodder were determined using Tilley and Terry (1963). Rumen fluid was collected from fistulated cow and dromedary camel before morning feeding that fed with a diet containing 60% forage and 40% concentrate for a month. The rumen fluid was flushed with CO_2 mixed and filtered through 4 layers of cheesecloth into the plastic tube and placed in a warm bath at 39°C. Then rumen fluid mixed with McDougall buffer (includes (g L^{-1}) 9.8 Sodium Bicarbonate, 2.44 Sodium Phosphate Dibasic, 0.57 Potassium Chloride, 0.47 Sodium Chloride, 0.12 Magnesium Sulfate and 0.16 Calcium Chloride) in a ratio 1:4. After flushed with CO_2, tubes were incubated at 39 °C for 48 h incubation; 6 ml of 20% HCl solution and 5 ml pepsin solution were added and then incubated for 48 h simulating post-ruminal degradation. After that, the residual substrates of each tube were filtered and used to determine the digestibility of DM, NDF and ADF, the digestibility was calculated by subtracting the amount of DM, NDF and ADF before and after incubation.

Statistical Analysis

The obtained data were analyzed as split plot design using General Linear Model (GLM) procedure on SAS software (SAS 2008), which is based on the following statistical model:

$$Y_{ijk} = \mu + P_i + \delta_{ik} + T_j + (PT)_{ij} + \varepsilon_{ijk}$$

Where Y_{ij} is observation, μ is the general mean, P_i is the effect of animal, T_i is the effect of treatment, PT_{ij} is the effect of treatment and animal, δ_{ik} is the standard error of main plot and ε_{ijk} is the standard error of term.

RESULTS AND DISCUSSION

The lowest digestibility of dry matter in dromedary camel (73.1%) and cattle (71.9%) was found in the treatment containing 100% fodder ($p<0.05$). But digestibility of NDF and ADF of diets containing different levels of Subabul instead of alfalfa were not different in cow and camel ($p>0.05$). Digestibility of NDF and ADF

Table 1. Ingredients and chemical composition of experimental diets were used for *in vitro* digestibility.

	Experimental diets			
Ingredients (%)	**Control**	**25%**	**50%**	**100%**
Alfalfa	18	9	4.5	-
Wheat straw	17	17	17	17
Wheat bran	12	12	12	12
Corn grain	25	25	25	25
Barley grain	13	13	13	13
Corn silage	14	14	14	14
Subabul leaves+pods+flowers	-	4.5	9	18
Salt	0.5	0.5	0.5	0.5
Mineral and vitamin supplement[1]	0.5	0.5	0.5	0.5
Chemical composition[2] (%)				
ME (Mcal kg^{-1})	2.30	2.35	2.36	2.35
CP	12.2	12.1	12.1	12.2
EE	3.2	4.9	5.2	5.5
NDF	33.4	35.3	36.5	38.2
ADF	19.1	19.3	20.2	21.5
ADL	4.07	4.5	4.6	4.87
Ash	5.02	5.08	5.13	5.18

[1] Provided the following (per kg of diet): Mn: 2,200 mg; Ca; 195 g; P: 80 g; Mg: 21,000 mg; Fe: 3,000 mg; Zn: 300 mg; Cu: 300 mg; I: 12 mg; Se: 1.1 mg; Co: 100 mg; 6,000 IU Vitamin A; 2,000 IU Vitamin D; 200 mg Vitamin E.
[2] ME: Metabolizable energy, CP: crude protein, EE: ether extract, NDF: Neutral Detergent Fiber, ADF: Acid Detergent fiber, ADL: acid detergent lignin.

in the treatment containing 100% Subabul in the cattle were 70.36% and 68.21%, respectively, and in the dromedary camel were 71.19% and 69.02%, respectively (Table 2).

Despite the high levels of protein, vitamins and minerals found in the Subabul leaves (Norton and Papi 1995), the digestibility and fermentation is decreased by 100% replacement, due to the presence of anti-nutrient compounds of Subabul leaves and pods (tannin, saponin, oxalate). Kang (2005) stated that the gas production decreased by the amount of lignin, tannin, oxalate and saponin in the diet. The amount of tannin of the Subabul leaves is more than other components (3.95%), which gas production and fermentation, can be increased by adding polyethylene glycol to leaves, due to the breakdown of bands between nutrients and tannins (Hassan Salam 2010).

Tannins can reduce the digestibility of nutrients by reducing the binding of microorganisms to particles (McAlister *et al*. 1994), inhibiting the growth of microorganisms and inhibiting of the activity of microbial enzymes (McSweeney *et al*. 2001). Researchers argued that the addition of saponin to alfalfa increased the efficiency of microbial protein synthesis in linearly, due to the negative effect of saponin on protozoa (Beauchemin *et al.* 2007). By decreasing the protozoa, the amount of bacterial predation by rumen protozoa decreases and leads to increases the microbial flow to the duodenum (Beauchemin *et al*. 2007).

Table 3 shows the effect of different diets containing Subabul on dry matter digestibility was significant ($p<0.05$). Treatments containing 50% and 100 % Subabul (74.38% and 70.37%) had lowest ($p<0.05$). The ADF digestibility in treatments containing 50% and 100% Subabul in compared with control treatment was 72.4% and 67.6% vs 73.5 %, respectively, and NDF digestibility

Table 2. The *in vitro* digestibility (%) of diets containing different levels of Subabul fodder instead of Alfalfa by using cow and camel rumen fluids (Animal and treatment effect).

Animal	Subabul	DM	NDF	ADF
Cow	0	76.8[b]	74.7	70.38
	25	75.2[c]	73.5	71.32
	50	76.1[b]	72.38	69.42
	100	71.9[e]	70.36	68.21
Camel	0	78.4[a]	74.71	72.8
	25	76.4[b]	74.2	71.6
	50	75.5[c]	72.91	72.18
	100	73.1[d]	71.19	69.02
SEM		0.2	4.2	3.1
p-value		0.04	0.2	0. 3

SEM = Standard error of means; in each column, values with different letters are significantly different ($p<0.05$).

Table 3. The *in vitro* digestibility (%) of diets containing different levels of Subabul fodder instead of Alfalfa by using cow and camel rumen fluids (Treatment effect).

	Digestibility		
Subabul	**DM**	**NDF**	**ADF**
0	77.02[a]	75.33[a]	73.5[a]
25	76.02[a]	75.1[a]	74.2[a]
50	74.38[b]	72.45[b]	72.4[b]
100	70.37[c]	69.65[c]	67.6[c]
SEM	0.2	0.6	0.8
p-value	0.02	0.05	0.04

SEM= Standard error of means; in each column, values with different letters are significantly different ($p<0.05$).

was 72.45% and 69.65% vs 75.33 % in treatments containing 50% and 100% Subabul and control, respectively ($p<0.05$).

Islam (1995) reported the amount of NDF and ADF of Subabul leaves and branches was 19.34% and 27.94%, and also stated that it can be used as a protein source equivalent to cottonseed meal in fattening ration.

In agreement with our results, Nasimi *et al.* (2016) reported *in vitro* digestibility of DM, NDF and ADF decreased with increasing Subabul leaves up to 75% in sheep. Paengkoum (2010) reported using Subabul leaves in diet decreased digestibility of DM and NDF. Nasimi *et al*. (2016) showed the nutrient digestibility reduced due to the greater content of lignin in Subabul leaves and branches than Alfalfa hay (11.04% vs. 9%) and anti-nutritional agents. However, levels of 25% and 50% of Subabul, due to the lower effect of anti-nutritional agents, lead to increase digestibility of DM, NDF and ADF. Jetana *et al.* (2012) reported that digestibility of nutrients was declined when diet containing 50–84 % Subabul fed with swamp buffalo and murah buffalo.

Table 4. The *in vitro* digestibility (%) of diets containing different levels of Subabul fodder instead of Alfalfa by using cow and buffalo rumen fluids (Animal effect).

	Digestibility		
Animal	**DM**	**NDF**	**ADF**
Cow	74.25[b]	70.5[b]	69.58[b]
Camel	76.6[a]	73.94[a]	71.4[a]
SEM	0.82	0.71	0.6
p-value	0.03	0.04	0.02

SEM = Standard error of means; in each column, values with different letters are significantly different ($p<0.05$).

The other reason for decreasing leaves digestibility at high levels of Subabul is the presence of saponin in the Subabul leaves that disrupt fiber digestion in the rumen, which may be due to decrease the activity of the fibrolytic enzyme (Lu 1987). Balgees *et al.* (2013) reported the degradability of Subabul leaves were less than the degradability parameters of Alfalfa. In a study, Shahriari *et al.* (2017) reported that degradability fractions were reduced by replacing alfalfa with Subabul pods.

Regardless of the type of treatment (Table 4), digestibility of dry matter, NDF and ADF in dromedary camel was more than cow ($p<0.05$). This increase of digestibility in camel can be attributed to the more activity of camel's foregut cellulolytic bacteria and enzyme production that play a very important role in increase digestion of fiber via growth of colonies on fibrous materials (Dadvar *et al.* 2018). Generally, researchers (Min *et al.* 2001) showed a greater ability for digestion of fibrous forage in the camels than other ruminants. This can be attributed to higher rumen retention time for the solid phase which facilitates cell walls degradation via a greater exposure time to microorganisms (Min *et al.* 2001). All reported digestive studies in camelids show that they have a special ability to use low quality forages. Gut cellulolytic activity in camelids is high because microorganisms have sufficient enzymatic activity to hydrolyze the cell wall carbohydrates and fermentation

of hydrolyzed oligosaccharides (Jouany *et al.* 1992). Kayouli *et al.* (1991) reported that dromedaries were able to digest low quality forage with higher yields than sheep that for straw, the activity of cellulolytic bacteria in camels was 20% higher than the other ruminants as sheep.

Researchers suggest that under the same conditions, camelids are the most adapted animals to the digestion of poor-quality forage than other ruminants living (Kayouli *et al.* 1991). It is showed when the low-quality roughages were fed; the microbial efficiency and the nutrient digestibility were significantly higher in llamas than sheep because of cellulolytic activity in the fore stomach of camelids was higher (Dulphy *et al.* 1997). But Kumar *et al*. (2002) reported that the number of rumen fungal in the cow was greater in diet based on rye-concentrate grass. Depending on the different sources, the digestibility between cows and other ruminants may differ according to the nutritional conditions of different diets (Bhatia *et al*. 2003).

CONCLUSION

According to the results of these experiments, substitution of 50% and 100% Alfalfa by Subabul branches (leaves+pods+flowers) in the cow and camel diet decreased *in vitro* digestibility. Therefore, due to the high price of protein source, it is recommended to use Subabul mixture of leaves+pods+flowers as a substitute for Alfalfa in the diet of cow and camel.

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