# The Economic Effect of a Daily Supplementation of carob pods (*Ceratonia siliqua* L.,) on Rumen Fermentation and Lactating Goats Performance.

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The present study was performed to investigate the effect of a daily supplementation of carob pods (*Ceratonia siliqua* L.,) on rumen fermentation and milk production of goats. Thirty two lactating does (weight ranged from 33–35 kg), aged 2-4 years old and from 2nd to 3th lactation season were randomly allocated into four similar groups (8 animals each). The animals were fed with isocaloric and isonitrogenous diets. Carob pods was daily supplemented at the rate of 0, 25, 50 or 100g /h/d. The lactating trial was extended for 75 days where goats were fed individually and fresh water was available at all time. Rumen fermentation parameters were monitored on three fistulated adult does. Results indicated that volatile fatty acids concentration, rumen volume, microbial protein synthesis and total bacteria counts were highest (P<0.05) with *C50* group compared with other groups. While, ammonia-N concentration and protozoa count were lower (P<0.05) with *C100* group compared with other groups. Milk production, protein and fat percentage were better (P<0.05) for *C50* and *C25* groups than those of *C100* group. Supplementation of Carob pods at 50 g caused a marked (P<0.05) increase in the enzymatic antioxidant activity (SOD, CAT, GPx, and GSH) but had a significant decrease (P<0.05) in TBARS compared to control group. Thus, it could be concluded that daily supplement of 50 g carob pods could be reasonable amount for goats performance without any adverse effect.

**Keywords**: carob pods; milk production; rumen fermentation; goats.

## Introduction

The carob tree (*Ceratonia siliqua* L.), is a leguminous evergreen tree native to the Mediterranean region, it's considered to be an important component of vegetation, economic and environmental reasons (Custodio et al., 2011). It cultivated for its edible seeds and pods, and it is used traditionally in livestock nutrition. Carob pod mainly consists of pulp (90 %), which is rich in sugars (48–56%), and in gross energy, making them a high-energy food in animal nutrition, but it also contains a large amount of condensed tannins (16–30 %) (Karababa and Coşkuner, 2013), although higher tannin values have been reported and low protein content (<50 g $kg^{-1}$ DM) (

Gasmi-Boubaker *et al*., 2008). However, the major negative effect results of tannins are resulted from either direct inhibition of digestive enzymes, or from formation of indigestible complexes with endogenous proteins. The presence of tannins in the rumen (pH 5.5-7.0) most plant proteins are bound and protected from microbial degradation, but are released in the abomasum (pH 2.5-3.51), enabling protein digestion and absorption of amino acids in the small intestine (Barry and Manley, 1984). Nevertheless, the presence of tannins in carob pods may have beneficial effects on human and animal health, due to their other properties, such as antidiarrheal, antibacterial, antioxidant and free- radical scavenging and antiproliferative activity in liver cells (Custodio *et al*.,

2011). So, the aim of this study was to investigate the economic effect of supplementing dietary goats with carob pods on their milk production and rumen fermentation activity was also studied.

**Materials and methods**

This experiment was conducted at Noubaria Experimental Station, Animal Production Research Institute, Agriculture Research Center, Egypt. Carob used in this experiment was obtained from local market, Alexandria, Egypt.

***Animals, diets and laboratory analyses***

Thirty two lactating Zaraibi does (2-4 years old, weighing 33-35 kg and from 2nd to 3th lactation seasons) were used in this experiment. They were divided into four equal groups (8 animals each) according to their age, initial live weight and number of kids. Before the start of the experiment, all does were kept 7 days for adaption, during which all animals were treated with Ivomec® injections against external and internal parasites. They were randomly assigned to four isocaloric and isonitrogenous experimental rations using a randomized complete block design (Steele and Torrie, 1980). Concentrate feed mixture (CFM) used consists of 33% yellow corn, 14% soybean meal, 20 % wheat bran, 20% barley grain,7% olive cake, 5% molasses, 2% limestone, 1.5%salt, 0.5% mineral-vitamin premix. Corn silages were fed *ad libtum* to goats.

Carob pods were daily supplemented at the rate of 0, 25, 50 or 100g /h/d, *C0, C25, C50 and C100*, respectively. Chemical composition of concentrate feed mixture (CFM) and carob pods are presented in Table (1).

**Table 1: Chemical composition of concentrate feed mixture (CFM) and carob pods (g/kg as DM).**

| Items | CFM | Carob pods |
|---|---|---|
| Dry matter | 891.5 | 942.6 |
| Organic matter | 941.6 | 963.7 |
| Crude protein | 138.5 | 61.6 |
| Crude fiber | 67.9 | 75.8 |
| Ether extract | 31.1 | 47.6 |
| NFE | 704.1 | 778.7 |
| Ash | 58.4 | 36.3 |
| NDF | 341.5 | 315.5 |
| ADF | 166.3 | 261.3 |
| ADL | 41.4 | 36.8 |
| Hemicellulose | 175.2 | 54.2 |
| Cellulose | 124.9 | 224.5 |
| Neutral detergent soluble | 658.5 | 68.4 |
| Total phenolic | 9.5 | 87.9 |
| Condensed tannins | 0.9 | 29.7 |

The lactating trial was extended for 75 days where goats were individually fed and fresh water was available at all the time. Milk yield was individually recorded for two successive days. Milk samples were collected 4 times in the 75 days twice daily according to Galatov (1994). Milk samples were chemically analyzed for total solid (TS), protein, fat and ash according to AOAC (2005), while lactose was calculated by difference.

***Rumen fermentation trials:***

Samples of rumen liquor were taken at 0,1, 3 and 6 h post feeding from three fistulated adult goats with approximately 30.5±0.5kg BW for each treatment, to be immediately analyzed for pH using Orion 680 digital pH meter. Rumen fluid samples were preserved for ammonia nitrogen ($NH_3$-N) determination according to Preston (1995), while concentration of total volatile fatty acid (TVFA's) was estimated by using steam methods (Warner, 1964). Microbial counts (bacteria and protozoa) in the ruminal fluid were determined using a counting cell (Hawskley, UK) as described by Demeyer (1981). Rumen volume was determined by the colorimetric method using Cr-EDTA according to El-Shazly *et al*. (1976). Microbial protein synthesized (MP g /day) in the rumen of goats fed the experimental diets was calculated using the model equation developed by Borhami *et al.* (1992):

g MP / day = mole VFA produced / day × 2 × 13.48 × 10.5 × 6.25 / 100

where: one mole VFA yield about 2 mole ATP (Walker, 1965), one mole ATP produce 13.48 $Y_{ATP}$ (g DM microbial cell) (Borhami *et al*., 1979), N % of dry microbial cell = 10.5 (Hungate, 1965).

***Antioxidant enzyme activities***:
Blood samples were collected at the end of the experimental period from the jugular vein of goats in the morning before access to feed and water. Plasma was obtained by centrifugation of blood and then stored at – 20 ºC until analysis. The concentration of SOD was determined by Sun and Sigma as described by Ogbunugafor *et al*., (2010). The catalase activity (CAT) was determined by the method of Beers and Sizer as described by Usoh *et al*., (2005). The activity of glutathione peroxidase (GPx) and reduced glutathione were determined by the method of Beutler *et al*. (1963). Lipid peroxide concentration measured as thiobarbituric acidreactive substances (TBARS) was performed according to the method of Trotta *et al*. (1982).

***Statistical Analysis:***
One–way analysis of variance was used to test the differences among the experimental groups. Means were separated by Duncan's Multiple Range test (Steele and Torrie, 1980). All statistical analyses were done using Proc ANOVA of statistical analysis system (SAS, 2004).

**Results and discussion**
***Ruminal fermentation:***
Ruminal pH values were not significantly affected by the dietary of different levels of carob pods supplementation (Table 2). We could notice effects of carob pods supplementation on its potential to prevent ruminal acidosis by reducing rapid starch hydrolysis, because feeding the carob pods diet did not create a severely acidotic fermentative environment in the rumen. While, concentrations of ruminal metabolites ($NH_3$-N and VFA's) were significantly (P< 0.05) varied among the experimental rations. *C100* had the lower $NH_3$-N and TVFA's concentrations, while *C50* was recorded the highest value of TVFA's concentrations compared with other groups. Molar proportion % of propionic acid was insignificantly lower with *C50* experimental rations. Acetic acid, acetic: propionic ratio and rumen volume (L) were higher (P<0.05) with *C50* rations. The effect of tannins on protein degradation is basically a reduction in the immediately degradable fraction, and a reduction of the fractional rate of degradation (Frutos *et al*., 2000). Condensed tannins are reported to reduce fibre, CP and OM digestibility because of their binding properties and inhibition of rumen microbes and also because CT itself are ruminally indigestible (Waghorn, 2008). Effects of carob pods on total VFA concentration and VFA pattern have been variable among studies depending on the dosage rate, the source of carob pods and its consists of condensed tannin. In contrast, Benchaar *et al*. (2008) and Aguerre *et al*. (2010) reported that total concentrations of VFA and molar proportions of individual VFA were not affected by feeding quebracho CT. The overall mean revealed that high (P < 0.05) rate of out flow from the rumen was obtained with goats fed *C100* compared to other rations. Average values of microbial nitrogen synthesis (MN) ranged from 15.01 to 17.57 (g/d) for *C100* and *C50*, respectively. The rate of out flow observed in this study with *C50* could be considered as suitable rate of out flow for efficient MN synthesis. Supplementation with carob pods at 50 g/d increased numbers of ruminal cellulolytic bacteria, which could increase silage degradability and increase the flow rate of microbial nitrogen as well and may alter the patterns of VFA's formations. Supplementation of carob pods significantly (P<0.05) decreased population of total Protozoa as compared with control. These results showed that the effects of carob pods could change population of rumen microorganism. The effect of carob pod on the cellulolytic activity of rumen microorganisms was shown to be correlated with its sugar rather than its tannin content. This is in accordance with experiments in which high sugar content was found to reduce cellulose digestion in the rumen (Bhat *et al*., 2013). The effect of tannins on ruminal protozoa count is variable in assays carried out in vivo (Makkar, 2003).

**Table 2: Effect of different levels of carob pods on rumen parameters of lactating Zaraibi does.**

| Items | *C0* | Carob pods | | | | |
|---|---|---|---|---|---|---|
| | | *C25* | *C50* | *C100* | SEM | P value |
| *pH* | *6.27* | 6.33 | 6.39 | 6.44 | 0.26 | 0.561 |
| *$NH_3$-N concentration (mg/100mlR.L)* | *14.88* $^{a}$ | 14.56 $^{ab}$ | 13.96 $^{b}$ | 12.83 $^{c}$ | 0.44 | 0.003 |
| *TVFA concentration (meq/100 mlR.L)* | *10.89* $^{b}$ | 11.16 $^{b}$ | 11.93 $^{a}$ | 9.94 $^{c}$ | 0.29 | 0.001 |
| *Acetic acid, %* | *54.93* $^{b}$ | 55.21 $^{b}$ | 56.69 $^{a}$ | 52.73 $^{c}$ | 0.61 | 0.001 |
| *propionic acid, %* | *23.98* $^{a}$ | 23.15 $^{b}$ | 22.51 $^{c}$ | 24.15 $^{a}$ | 0.36 | 0.008 |
| *Acetic : propionic ratio* | *2.29* $^{b}$ | 2.38 $^{b}$ | 2.52 $^{a}$ | 2.18 $^{c}$ | 0.09 | 0.006 |

| Items | *C0* | Carob pods | | | | |
|---|---|---|---|---|---|---|
| | | *C25* | *C50* | *C100* | SEM | P value |
| Rumen volume (L) | 3.14 c | 3.29 b | 3.48 a | 3.02 d | 0.07 | <0.0001 |
| Rate of out flow (%h) | 6.06 b | 5.98 c | 5.61 d | 6.27 a | 0.04 | 0.0001 |
| *Microbial N yield (g /d).* | *16.22 c* | 16.69 b | 17.57 a | 15.01 d | 0.21 | 0.0001 |
| Total bacteria counts, $\times 10^8$ cfu/ml | 1.15 b | 1.19 b | 1.28 a | 1.01 c | 0.04 | <0.0001 |
| Total protozoa counts, $\times 10^6$cfu /ml | 4.61a | 3.88 b | 3.71 b | 3.43 c | 0.21 | 0.001 |

*a, b, c and d: means in the same row with different superscripts are significantly (P<0.05) different.*

***Feed Intake, milk yield and milk composition:***

Averages of daily dry matter intake by Zaraibi goats during the experimental periods are summarized in Table 3. Less (P<0.05) feed intake was recorded for *C100* group than other groups they had more (P<0.05) feed intake without insignificant (P>0.05) effect between each other. However, no significant effect (P<0.05) among rations for C/R ratios. The reduction in feed intake for *C100* group could be explained by its more tannins content which can act as antinutritional factor, whereas it had the capacity of reducing the digestibility of proteins in the ration (Mariscal-Landin *et al*., 2004). According to Kotrotsios *et al.* (2010) carob pods inclusion in pig diets significantly reduced the digestibility of proteins, fats, fibers and minerals, especially in the weaning and growing periods. The action of tannins on animals probably depends on their solubility, in the gastrointestinal tract (Serrano *et al*., 2009). This depress effect would be attributed to degraded palatability or to a short-term effect of astringency (Landau *et al*., 2000). Higher (P<0.05) milk yield, milk fat and milk protein were found for *C50* group followed by *C25* and control groups, while the lowest one was for *C100* group. This could be due to the less feed intake, digestibility and the capability of CD on reducing the efficiency of protein in the ration compared to other groups. This resulted in less (P<0.05) milk fat and milk protein than other groups. These data was confirmed by Grainger *et al*.( 2009) who reported that milk yield, fat and protein proportion of milk could be reduced if dairy cows fed condensed tannins daily. This contrasts with Wang *et al*. (1996) who illustrated that tannins from Lotus *corniculatus* fed to lactating ewes increased milk yield, lactose and protein contents. The explanation of these effects could be related to the increase in metabolizable protein supply which binded to condensed tannins, where it protected from microbial degradation, and then released in the abomasums, enabling protein digestion and absorption of amino acids in the small intestine (Barry and Manley, 1984). However, the effects of tannins on ruminant productivity depend on the quality and quantity of dietary protein (Patra and Saxena, 2011).

Table 3: Dry matter intake, milk yield and milk composition of lactating Zaraibi does fed the experimental rations.

| Items | C0 | Carob pods | | | | |
|---|---|---|---|---|---|---|
| | | *C25* | *C50* | *C100* | SEM | P value |
| ***Dry matter Intake, g/d***: | | | | | | |
| *CFM intake* | 752.41 a | 756.36 a | 779.63 a | 675.15 b | 0.25 | 0.004 |
| *Corn silage intake* | 397.38a | 388.22 a | 402.16 a | 360.44 b | 0.16 | 0.007 |
| TDMI | *1149.79 a* | 1144.58 a | 1181.79 a | 1035.59 b | 40.18 | 0.002 |
| Concentrate : roughage ratio (C:R) | *65.44: 34.65* | 66.08: 33.92 | 65.98: 34.02 | 65.19: 34.81 | | |
| ***Milk Production*** | | | | | | |
| Milk yields, kg/day | 0.856 c | 1.002 b | 1.075 a | 0.721 d | 0.42 | 0.002 |
| Milk fat, g/day | 34.84 b | 43.59 a | 48.16 a | 27.76 c | 4.65 | 0.0004 |
| Milk protein, g/day | 25.68 b | 31.56 a | 34.72 a | 19.68 c | 3.99 | 0.0002 |
| ***Milk composition (%) :*** | | | | | | |
| Total solids | 11.88 b | 12.13 a | 12.19 a | 11.84 b | 0.12 | 0.003 |
| Solids not fat | 7.81 b | 7.78 b | 7.71 b | 7.99 a | 0.11 | 0.003 |
| Fat | 4.07 c | 4.35 b | 4.48 a | 3.85 d | 0.09 | <0.0001 |
| Protein | 3.00 b | 3.15 a | 3.23 a | 2.73 c | 0.08 | 0.004 |
| Lactose | 4.10 b | 3.90 c | 3.74 c | 4.52 a | 0.17 | 0.001 |
| Ash | 0. 71 | 0.73 | 0.74 | 0.74 | 0.08 | 0.257 |

*a, b, c and d: means in the same row with different superscripts are significantly (P<0.05) different.*

***Efficiency of milk production and economic evaluation:***

When milk efficiency was expressed as DMI per kg 4% FCM produced, *C50* group was more efficient, followed by *C25* (Table 4), while the least efficiency one was found with C100 group. The control group showed less feed cost to produce one kg milk followed by *C25* and *C50* groups. The economic return (L.E. / h/ d) or the profit above feeding cost was higher with carob pods daily supplemented (*C50* and *C25)* than other rations.

Table 4: Nutrients intake, feed conversion and economic evaluation of daily milk production of goats fed the experimental rations

| Items | C0 | Carob pods | | | SEM | P value |
|---|---|---|---|---|---|---|
| | | *C25* | *C50* | *C100* | | |
| ***Nutrients intake(kg/h/d):*** | | | | | | |
| DMI, g | *1149.79* [a] | 1144.58 [a] | 1181.79 [a] | 1035.59 [b] | 40.18 | 0.002 |
| 4% FCM*, kg | 0.87 [b] | 1.06 [a] | 1.14 [a] | 0.71 [c] | 0.07 | 0.0001 |
| ***Feed conversion ( kg / kg ):*** | | | | | | |
| *DMI / FCM* | 1.32 [b] | 1.07 [c] | 1.04 [c] | 1.46 [a] | 0.08 | 0.0008 |
| ***Economic evaluation**:*** | | | | | | |
| Daily feed cost, L.E | 2.29 | 2.55 | 2.87 | 3.07 | | |
| Price of daily milk yield, L.E | 4.28 | 5.01 | 5.38 | 3.61 | | |
| Economic return, L.E | 1.99 | 2.46 | 2.51 | 0.54 | | |
| Economic return,(h/d)% | 100 | 123.62 | 126.13 | 27.14 | | |

[abc] means in the same row with different superscripts are differ significantly (P< 0.05).

*Fat correct milk (4%) was calculated according to Gaines (1923) using the following equation: FCM = 0.4 M + 15.0 F, Where M = milk yield and F = fat yield

* *Calculation based on the following price in Egyptian pound (L.E.) per ton at 2009, concentrate feed mixture (CFM) =2500 L.E/ton, corn silage =300 L.E/ton, carob pods =5 L.E/kg, and One kg of raw milk 5 L.E/kg.

***Antioxidant enzymes activity***:

Data in Table (5) indicated that supplementation with different levels of carob significantly ($p$<0.05) increased SOD, CAT, GPx and GST activities and decreased TBARS in plasma compared to the control group. This revealed that supplementation with carob pods prevented the lipid peroxidation by enhancing the SOD, CAT and GPx activities. It is well known that many phenolic compounds, which are found in carob, exert powerful antioxidant effects. They, also, inhibit lipid peroxidation by scavenging reactive oxygen species (ROS), such as OH (Shahidi and Wanasundara, 1992). Polyphenols in carob pods have antioxidant activity (Kumazawa *et al*., 2002). In addition the crude polyphenol extracts of carob pods showed strong antioxidant activity (Harbore,and Baxter, 1999). The prevention of lipid peroxidation might, at least in part, be derived from the capability of carob to scavenge ROS, which was supported by the observation that carob reduced the level of TBARS production (Hsouna *et al.*,2011). When rats were treated with carob the reduction of SOD, CAT and GPx activities was inhibited, which protected against factors of oxidative stress, in particular $H_2O_2$. The reduced activity of SOD, CAT and GPx could be due to enhanced lipid peroxidation or inactivation of the antioxidative enzymes (Klenow *et al.,* 2009). The antioxidant activity caused by the presence of these compounds could have additional effects, sparing other antioxidants and protecting molecules from oxidative damage during digestion and preserving the intestinal epithelium from potential oxidative damage caused by dietary factors or bacterial metabolism (Goni and Serrano, 2005).

**Table 5: Plasma antioxidant enzymes activity in lactating Zaraibi does fed different levels of carob pods**

| Items | C0 | Carob pods | | | SEM | P value |
|---|---|---|---|---|---|---|
| | | *C25* | *C50* | *C100* | | |
| | 7.01 [c] | 8.49 [b] | 8.81 [b] | 9.59 [a] | 0.32 | 0.007 |
| CAT | 9.06 [b] | 9.76 [a] | 10.08 [a] | 10.24 [a] | 0.42 | 0.016 |
| GPx | 6.22 [b] | 7.24 [a] | 7.56 [a] | 7.74 [a] | 0.55 | 0.022 |
| GST | 7.59 [c] | 8.33 [b] | 8.48 [b] | 9.14 [a] | 0.29 | 0.001 |
| TBARS | 1.96 [a] | 1.42 [b] | 1.36 [b] | 1.28 [b] | 0.17 | 0.025 |

[a, b and c]: means in the same row with different superscripts are significantly (P<0.05) different.

Superoxide dismutase (SOD U/ml); catalase (CAT; µmol $H_2O_2$ consumed/min./ml), glutathione peroxidase (GPx; U/ml), glutathione S-transferase (GST; µmol/hr/ml) and thiobarbituric acid-reactive substances (TBARS; nmol/ml),

## Conclusion

In conclusion, supplementing goats with 50 g/d of carob pods can be promising in preventing them from any undesirable of antioxidants status, not only that but enhanced the utilization of the ration to get more milk yield and better an economic results without any adverse effect.

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