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Effect of partial dietary substitution of Carob (Ceratonia siliqua L.) to barley grains on diet digestibility in growing rabbits.

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Abstract - The present study aims to evaluate the effect of the substitution of carob pods to barley, on apparent nutrient digestibility of rabbit feeds. A total number of 30 male rabbits of 7 weeks of age and with a live body weight of 1116 ± 118 g were randomly allotted to 3homogeneous groups (10 rabbits/group, 1 rabbit/cage). Each group received one of the experimental diets Control, Carob 10 and Carob 20, containing 0, 10 and 20% of carob pods, respectively. The assay was conducted, according to the European reference method reported by Perez et al. (1995). Digestibility of dry matter (DMD), organic matter (OMD), crude protein (CPD), crude fiber (CFD), neutral detergent fiber (NDFD), acid detergent fiber, (ADFD) and hemicelluloses (HCD) were determined. The results showed that carob pods reduced diet digestibility in rabbits. This effect can be attributed mainly to its relatively high lignocellulosic content. Indeed, if the incorporation rate reached20 %, the Acid Detergent Fiber and Acid Detergent Lignin content would increase, respectively, to 23 and 14 % DM.

Keywords: Carob, Rabbits, apparent digestibility

1. Introduction

In Tunisia, animal feeds industry is increasingly reliant on mainly imported raw materials, sub-products and ingredients such as barley grains, maize, soybean meal and dehydrated alfalfa (OEP, 2018). Moreover, due to substantial increase in international market price, their prices increased significantly, which put economical pressure on livestock owners (Blache et al., 2008 ; Obeidat et al., 2011). In order to overcome this issue, the use of local ingredients, such as carob pods, could to be a good alternative and reliable feed resource (Lounaouci-Ouyedet al., 2014). Carob tree (*Ceratonia siliqua* L.) is widely used in the semi arid Mediterranean regions. It belongs to *Cesalpiniaceae* sub-family of the *Leguminosae* family (Battle and Tous, 1997; Yousif and Alghzawi, 2000 ; Biner et al., 2007). This species can survive long drought periods and it adapts well to poor soils (El Batal et al., 2013 ; El Batal et al., 2016). It is cultivated for ornamental, medicinal, feed uses (animal and human) and industrial purposes (Girolamo and Laura, 2002 ; Hadj Salem, 2004).Nowadays, the renewed interest in this tree has allowed its prosperity in the Mediterranean countries (Karachi, 2008). The pulp of carob pods (fruit of *Ceratonia siliqua* L.) contains high contents of sucrose, fructose, glucose and fiber (El Batal et al., 2016, Rtibi et al., 2017). Carob pods were used in livestock nutrition, as an alternative to substitute barley grains in sheep diets (Karabulut et al., 2006), poultry (Ortiz et al., 2004) and rabbits (Gasmi-Boubaker et al., 2008). This study aims to determine the effect of substituting partially barley grains with local carob pods on nutrients' digestibility in rabbits' diets.

2. Material and methods

2.1. Diets

A basal diet containing 40.60% of barley grains, 10% of soybean meal, DDGS maize 10% and dehydrated alfalfa 32%. Two other diets, Carob 10 and Carob 20 were formulated, in which 10% and 20% of carob pods substituted barley grains. All the diets were supplemented with calcium carbonate 0.2%, monocalcium phosphate 0.2%, sodium chloride 0.5% DL-methionine 0.05, L-lysin and 0.5% of vitamin-mineral premix.

2.2. Animals and digestibility trial

All procedures were achieved under Law No. 2005-95 of the Tunisian Agriculture and Water Resources Minister for livestock and animal products. A total number of 30 New Zealand male rabbits of 7-week-old of age an average live body weight of 1116 ± 118 g were used in the essay. Animals were randomly divided into three homogeneous groups and housed individually in flat-deck wired cages (76×45×25 cm). Cages were designed to allow individual feces collection and feed intake control. Each group was fed one of the experimental diets (Control), (Carob 10) and (Carob 20). Digestibility trial was conducted according to the European reference method developed by Perez et al. (1995). The experiment was conducted over a total duration of 12 days: 7 days of adaptation (including adaptation to the new environment) and 5 days of feed intake and excreted feces collection data and samples. Feces samples were collected daily and stored at (-20°C) for subsequent chemical analysis. Feed intake (g), Metabolic feed intake (g/kg $LW^{0.75}$)and the coefficients of total tract apparent digestibility (CTTAD) for Dry matter (DM), organic matte (OM), crude protein (CP), crude fiber (CF) gross energy (GE), neutral detergent fiber (NDF), acid detergent fibre (ADF) and Hemicelluosewere calculated according to the methodology described by Perez et al. (1995).

2.3. Chemical composition

Feed samples and feces were prepared for chemical analysis according to the recommendations of Perez *et al.* (1995). Dry (DM) and organic (OM) matter, Crude fiber (CF) and Crude protein (CP) were analyzed according to AOAC (2000). Neutral (NDF), acid (ADF), detergent fiber and lignin (ADL) of diets and feces were analyzed according to Van Soest (1991) methods. Hemicellulose were calculated according to the methodology described by Perez et al. (1997) and condensed tannins (CT) were analyzed by using the vanilline-HCl method, reported by Makkar and Becker (2001).

2.4. Statistical analysis

The data were analyzed by one-way analysis of variance using the GLM procedure of SAS (2003). An analysis of the correlation within variables (the digestive utilization coefficients and the chemical composition of food) in pairs was performed, and then the Stepwise procedure was used to establish prediction equations. All differences were considered to be significant at $P \leq 0.05$. Regression figures between carob feed substitution to barley grains and diet digestibility were established with Excel program (office 365).

3. Results and discussion

3.1. Chemical composition of feeds

Guenaoui et al (2019) reported lower CP, ADF and ADL content and higher NDF content than our results for carob pods.In comparison with the growing rabbit requirements cited by De Blas and Mateos (2010), the three diets (Table 1) provided all the nutrients necessary for the growth and development of animals, with an excess of fiber and protein.

Table 1. Feed and carob pods components (%) of the diets

	Control	Carob 10	Carob 20	Carobpods	Barley grains
Barley	40.60	30.60	20.60	-	100
DDGS Maize	10.00	10.00	10.00	-	-
Soybeanmeal	16.00	16.00	16.00	-	-
Alfalfahay	32.00	32.00	32.00	-	-
Carobpods	0.00	10.00	20.00	100	-
Calcium carbonate	0.20	0.20	0.20	-	-
Monocalcic phosphate	0.20	0.20	0.20	-	-
Sodium Chloride	0.50	0.50	0.50	-	-
DL-Methionine	0.05	0.05	0.05	-	-
L-Lysine	0.02	0.02	0.02	-	-
Premix [(1)]	0.43	0.43	0.43	-	-
DE (kcal/kg)[(2)]	2422	2305	2187	1850	3300
DM	92.87	93.02.	92.55	86.50	93.60
OM (%)	93.69	94.02	95.40	96.90	97.02
CP (% DM)	17.32	16.70	16.09	5.30	12.4
CF (%DM)	12.93	13.14	13.35	8.96	9.0
NDF (%DM)	28.37	29.98	31.59	28.26	19.10
ADF (%DM)	17.10	19.69	22.28	27.00	7.20
ADL (%DM)	4.53	6.54	8.55	18.70	1.20
Hemicellulose(%DM)	18.90	15.00	13.70	1.30	11.90
Cellulose (%DM)	13.40	7.20	8.70	8.30	6.00
Condensed tannins (eq. catechin % DM)	0.054	0.073	0.092	0.19	ns

[(1)]Premix Composition contains (g/kg premix): Zn, 7; Mg, 5; Mn, 5; Cu, 2.3; Fe, 3.4; I, 0.12; Se, 0.025; Co, 0.03; thiamine, 0.05; riboflavin, 0.04; folicacid 0.4; vitamin K3, 0.2; biotine, 0.0015; vitamin A, 850000IU, Vitamin D3,175000 IU; Vitamin E 1500 IU

[(2)] Estimated energy according to FEDNA tables (De Blas et *al.*, 2003).

*[(3)]Race

Carob incorporation resulted in a slight reduction in protein content of the Carob 20 feed and increasing in ADF and ADL components. Indeed, Carob 10 and Carob 20, contained higher concentrations, while hemicellulose and cellulose were lower than in control diet.
The incorporation of 10 and 20% of carob with 0.193 g eq catechin/100g DM of CT, increased condensed tannins from 0.054 g (eq catechin/100g DM) in control diet to 0.073 and 0.092 g (eq catechin/100g DM) content in carob pods, respectively.

3.2. Total tract apparent digestibility

The data on the feed intake and the CTTAD of the experimental diets are presented in Table 2. The DM intake (P = 0.319) and the metabolic intake (P = 0.115) were not affected by dietary treatment. However, Gasmi-Boubaker et al. (2012) reported that the feed intake was higher in rabbits fed the commercial diet than in other rabbit's diets, where carob pods was incorporated at 8 % and 15 %. Abu Hafsa et al. (2017) founded that at 5% of carob pods inclusions enhanced the daily feed intake of rabbits but a significant decrease was reports at level of 10% of carob pods.

Table 2. Effect of carob incorporation's on feed intake and coefficients of total tract apparent digestibility of rabbits

	Control	**Carob10**	**Carob20**	**SEM**	**P-value**
DM intake (g/d)	115	124	120	3.970	0.319
Feed metabolic intake (g/kg of $LW^{0.75}$/d)	95	103	105	3.317	0.115
CTTAD					
DM	65.53^{a}	62.54^{a}	58.61^{b}	1.013	0.0005
OM	67.20^{a}	64.00^{b}	59.82^{c}	0.987	0.0002
CP	79.66^{a}	72.71^{b}	66.00^{c}	1.270	<0.0001
CF	15.36^{a}	18.47^{a}	9.65^{b}	1.291	0.001
NDF	36.58^{a}	25.87^{b}	26.09^{b}	2.078	0.005
Cellulose	22.09^{ab}	20.42^{b}	26.79^{a}	1.481	0.048
Hemicellulose	49.31^{a}	32.61^{b}	30.90^{b}	2.191	<0.0001

DM: Dry matter; OM: Organic matter; CP: Crude protein; CF: Crude fiber; NDF: Neutral detergent fiber; ADF: Acid detergent fiber; ADL: Acid detergent lignin
SEM : Standard error mean
[a,b,c] : Means in the same line with different superscripts differ significantly at $P \leq 0.05$.

In contrast, nutrient digestibility showed differences among dietary groups. A decrease of the CTTAD of Dry matter and crude fiber, was recorded in carob20, whereas in carob 10, the CTTAD was similar to control diet (P = 0.05 and P = 0.12, respectively for DM and CF).
On the other hand, OM and CP digestibility decreased with the inclusion of the carob (P <0.03), respectively for OM and CP. This decrease was of the order of 3.2 points and 4.18 points for the OM and 6.95 points and 6.71 points for the CP, respectively for the Carob 10 and Carob 20.Furthermore, Carob 10 and Carob 20 diets lots had similar NDF and hemicellulose digestibility (P > 0.61), but much lower than those of Control lot (25.98 *vs* 36.58 and 31.75 *vs* 49.31 for NDF and hemicellulose respectively, P <0.004).Abu Hafsa et al. (2017) reported a significant effect on the apparent digestibility of nutrients of the different inclusion levels of carob pods. Indeed, DM, CP and CF digestibility were higher at 5% of carob pods addition's but lower at 10% of carob pods inclusion rate in the diet.
The reduction of digestibility of different nutrients can be attributed to several causes. Carob pods induced higher contents in fiber components, in particular lignin and high lignin / cellulose ratio (11.4 and 14.4 respectively for Carob 10 and Carob 20) causing the decrease of DM and CP digestibility (Gidenne et al., 2011). Moreover, carob is characterized by the presence of tannins, in particular condensed tannins(0.193 g eq catechin/100g DM), which had the property of binding to proteins rich in the gastrointestinal tract of the animals, interfering with the digestion and absorption of carob proteins and nutrients (Lizardo et al., 2002; Serrano et al.,2009). Mariscal-Landin et al. (2004) explained that tannins could inhibit digestive enzymes by precipitating proteins and therefore interfere with the digestion and absorption of nutrients.
Nevertheless, Gasmi-Boubakar et al. (2008) showed an improvement in the digestibility coefficients of DM and OM using Tunisian carob variety, but replacing the entire control feed with carob pulp. This difference is probably due to a variation in feed chemical composition.
In order to confirm the presented hypotheses, an analysis of two-by-two variables made it possible to establish a correlation matrix. Negative correlations were observed between the DM, OM and CP CTTAD and ADF and ADL contents (-0.873 <R <-0.658). However, The DM, OM and CP CTTAD were positively correlated with the cellulose level (0.546, 0.563 and 0.678, respectively). Digestibility of CF, NDF, ADF and hemicellulose showed moderate to low correlations with OM and CP

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concentrations (0.486 <R <0.672). The ADF and HC CTTAD were negatively correlated with ADF and ADL concentrations, and the CF CTTAD was negatively correlated with NDF content (R = - 0.705).
The results of the prediction equations of the coefficients of total tract apparent digestibility are reported in Table 3. The prediction of the $CTTAD_{DM}$, $CTTAD_{MO}$ and $CTTAD_{CP}$ of feeds was allowed by the lignin / hemicellulose ratio (ADL / HC) with regression coefficients (R^2) ranged from 0.69 to 0.75 (p <0.001). The NDF and HC fiber digestibility prediction equations also showed high R^2 (0.66 and 0.77, respectively).

Table 3. Significant prediction equations of CTTAD's based on chemical composition

Y variable	Equation	R^2
$CTTAD_{DM}$	67.479 – 7.179* ADL/HC	0.69***
$CTTAD_{OM}$	69.261– 7.711* ADL/HC	0.73***
$CTTAD_{CP}$	82.840 – 14.996* ADL/HC	0.75***
$CTTAD_{CF}$	103.711 – 2.527* NDF	0.49**
$CTTAD_{NDF}$	13.007– 1.802* Cellulose	0.66***
$CTTAD_{ADF}$	60.238 – 67.994* CP/NDF	0.34*
$CTTAD_{HC}$	57.167 – 39.140* ADL/ADF	0.77***

CTTAD: coefficients of total tract apparent digestibility; DM: Dry matter; OM: Organic matter; CP: Crude protein; CF: Crude fiber; NDF: Neutral detergent fiber; ADF: Acid detergent fiber; ADL: Acid detergent lignin; HC=Hemicellulose
*p<0.05 ;**p<0.01 ; p<0.001

Thus, the use of carob has a negative incidence the digestibility of diets by decreasing their concentration of protein and digestible fiber and by increasing their lignocellulose content.
Figures 1, 2, and 3 representing the variation of the DM, OM and CP digestibility coefficients with the rate of incorporated carob, showed high regression coefficients (0.66 <R^2 <0.74, p ≤ 0.0001).

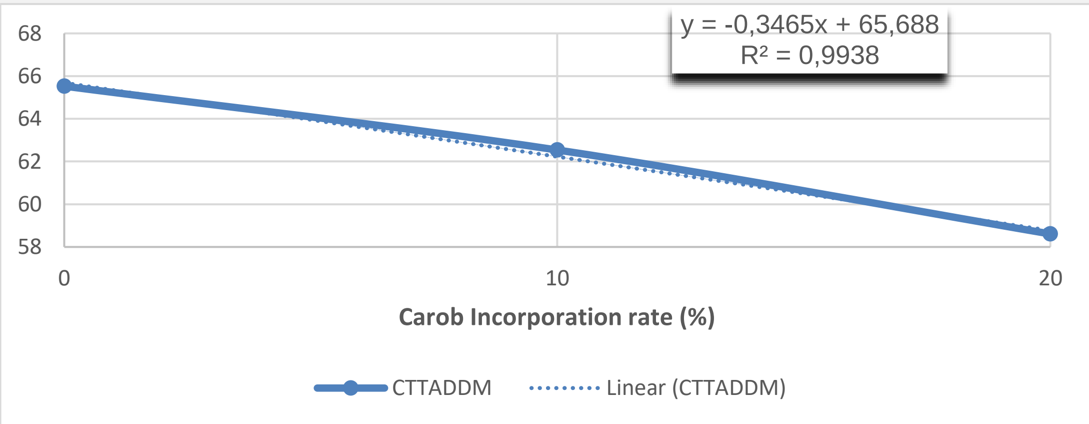

Figure 1. Variation of coefficients of total tract apparent digestibility of dry matter with carob rate incorporation

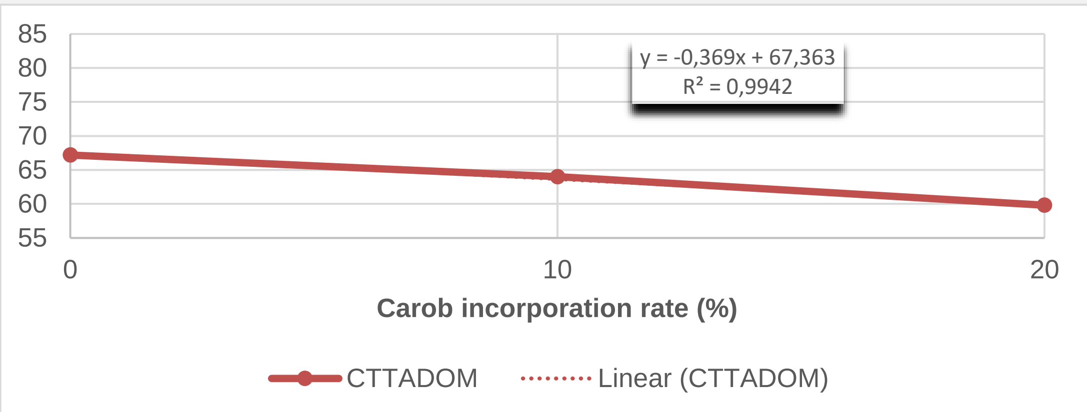

Figure 2. Variation of coefficients of total tract apparent digestibility of organic matter with carob rate incorporation

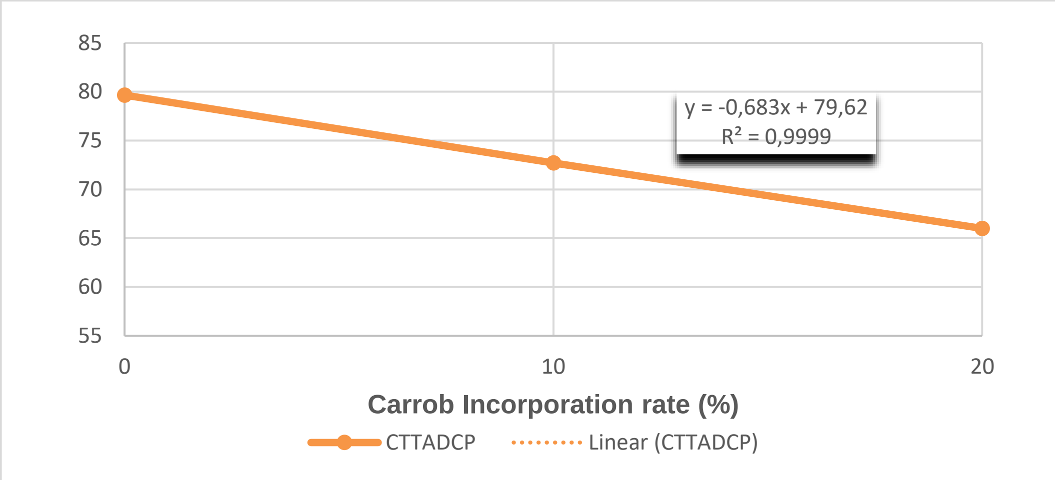

Figure 3. Variation of coefficients of total tract apparent digestibility of crude protein with carob rate incorporation

4. Conclusion

Thus, the carob is a local resource that represents a good alternative to imported raw materials, to reduce feeding cost in Tunisian rabbit farms. The substitution of carob with barley grains has no incidence on feed intake, and dry matter digestibility was not affected with the inclusion of 10% carob in the growing rabbit feed. However, by replacing 10 or 20 % of barley grains with carob pods in rabbit diets decreased CP, NDF, ADF, hemicellulose and cellulose digestibility. Thus the nutritional value and chemical composition of carob and the total diet should be considered in order to keep a balanced rate of fiber, especially ADF and ADL. Further research can study the effect of incorporating lower inclusion ratio of carob pods in feeds on rabbit gut microflora, ceacal fermentation and intestinal health and morphology.

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