

INFLUENCE OF DAILY SUPPLEMENTATION OF CARIB PODS (*Ceratonia Siliqua* L.) AS POLYPHENOL RICH PLANTS ON RUMEN FERMENTATION AND LAMBS GROWTH PERFORMANCE

Mohamed Samir KHALEL¹, Mohamed Helmy YACOUNT¹,
Ayman Abdel-Mohsen HASSAN¹, Amr Mohamed SHWERAB¹,
Dorina MOCUTA², Adrian TUREK-RAHOVEANU²

¹Animal Production Research Institute, Agriculture Research Center, Department of by-products Utilization, Dokki, Giza, Egypt, Emails: drmohamedes@yahoo.com; helmy_yacout555@hotmail.com; aymanan19@hotmail.com; amr.shwerab@yahoo.com

²University of Agricultural Sciences and Veterinary Medicine, Faculty of Management and Economic Engineering, Bucharest, 59 Marasti Boulevard, District 1, 011464, Romania, Email:dorinamocuta@yahoo.com

Corresponding author: aymanan19@hotmail.com

Abstract

*This study was conducted to evaluate the influence of daily supplementation of carob Pods (*Ceratonia Siliqua* L.) at the rate of 0, 25, 50 and 100 gm on digestibility coefficients, rumen fermentation, degradation kinetics, microbial protein synthesis, some blood parameters, antioxidant activity and lambs growth performances. Three rams were consigned for the digestibility trials, while other three females with rumen fistula were used for the in situ trials. Fourty lambs (6-8 months) were used for feeding trials (90 days). Data showed that daily supplement with 50gm carob pods had higher digestion coefficients, lower rumen NH₃-N, but higher TVFA's concentration, and microbial-N synthesis compared with other supplementation rats. In addition to more effective degradability of DM and CP, feed and economic efficiencies and daily weight gain. Supplementation with 100 g carob pods was the worst one. Insignificant differences were found for almost blood constituents among the experimental diets, they were in the normal ranges. So, it could be concluded to daily supplement sheep with 50gm of carob pods in order to achieve good productive performance of lambs. However, it still needs to carry long term trials on the field of meat or milk production.*

Key words: carob pods, digestibility, rumen fermentation, degradation kinetics growing lambs, blood constituents

INTRODUCTION

Carob tree (*Ceratonia siliqua* L.) is native to the Mediterranean area, the main producers of carob fruits are Spain, Italy, Portugal, Morocco and Greece [23]. Traditionally, carobs were cultivated for human and animal nutrition, while nowadays carob seeds and pods have a wide application as natural food additives and stabilizer agents in the food industry, e.g. cocoa substitute, gums, sugars, beverages or pharmaceutical and cosmetic industries [55]. However, the presence of tannins in carob pods with their properties such as antidiarrheal, antibacterial, antioxidant and free-radical scavenging and antiproliferative activity in liver cells may have some beneficial effects on human and

animal health [12] [16]. Carob pods have been a wide used in animal nutrition, for sheep [33], lambs [47], rabbits [26], and poultry [44].

Tannins, can be divided according to their chemical structure into four major groups: condensed tannins, hydrolysable tannins, phlorotannins and complex tannins [50]. They had the ability to form insoluble complexes with proteins or digestive enzymes which reduce digestibility of dietary proteins [31]. On the other hand, dietary tannins appear to have some other beneficial effects in ruminants, they mainly prevent excessive ruminal degradation of dietary proteins, reduce the activities of microbial proteases [8]. The present paper aimed to evaluate the effect of four supplementation rates (0, 25, 50

and 100 gm.) of carob pods on digestibility, rumen fermentation, degradation kinetics, some blood constituents and lambs performance.

MATERIALS AND METHODS

A total of 40 male growing lambs (8 months) with an average weight of 24.56±0.13 kg were randomly allotted into 4 groups (10 animals each) to study their growth performance. Four experimental concentrate feed mixtures (CFM) were formulated with 14 % CP. Lambs were fed the CFM's and rice straw (RS) and daily supplemented with 0 or 25, 50 or 100 mg carob pods for 90 days according to [42]. A digestibility trial was conducted by 12 adult rams (3 rams for each group), they were individually allotted in metabolic cages for 3 weeks as adaptation period followed by 7 days for collecting urine and feces. Animals were fed as above at 8.00 am and 4.00 pm., while water was available all over the day. Table 1 illustrated composition of CFM and its chemical composition and carob pods.

Feces and urine were collected quantitatively once a day before the morning meal at 8.00 am and weight fresh. Daily samples representative 20 % of fresh feces and urine, then the seven days combined collection of samples were stored at - 20 °C and kept for routine analysis. Feeds and fecal samples were analyzed for crude protein (CP) by Kjeldahl, crude fiber (CF), ether extract (EE) and ash, while urine was analyzed for nitrogen (N) according to AOAC [4]. Cell wall (neutral detergent fiber (NDF), acid detergent fiber (ADF), acid detergent lignin (ADL), cellulose and hemicelluloses content was determined by [58] procedure. Values of the total digestible nutrients (TDN) were calculated according to the classic formula of Cheeke on a dry matter basis [15].

Live body weight (g) and daily feed consumption (g) were individually recorded. Feed conversion was calculated as feed consumption (g) / weight gain (g). Economical efficiency (Y) was calculated according to the following equation: $Y = (A - B) / B \times 100$, where (A) is the selling cost of

the obtained gain and (B) is the feeding cost of this gain.

Table 1. Composition of CFM & its chemical analysis and carob pods (on DM basis).

Ingredients (g/kg)	CFM	Carob pods
Yellow corn	330	
Barley grain	200	
Wheat bran	170	
Soybean meal (44 % CP)	140	
Olive cake	70	
Molasses	50	
Limestone	20	
Sodium chloride	15	
Mineral-vitamin premix ²	5	
Chemical analysis (g/kg DM):		
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 ¹	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
Total tannins	0.9	29.7

One kilogram of premix contain: Vit. A 12000 000 IU, Vit. D3 2200 00 IU, Vit. E 1000mg, Vit. B1 1000 mg B2 4000 mg, Vit. B6 100 mg, Vit. B12 10 mg, Pantothenic acid 3.33 g, Biotin 3mg, Folic acid 0.83 g, Zn 11.79 g, Mn 5 g, Fe 12.5 g, Cu 0.5 g, Se 16.6 mg and Mg 66.7 g.

¹NFE = 100 – (% CP+ % EE + % Ash + % NDF)

Source: Own results.

Rumen fermentation trials

Three fistulated Barki female sheep (45.5±0.5kg BW) were used for rumen fermentation trials. Samples of rumen liquor were taken at 0, 1, 3 and 6 h post feeding from for each treatment, to be analyzed for pH using Orion 680 digital pH meter. Rumen fluid samples were preserved for ammonia nitrogen (NH₃-N) determination according to Preston [46], total volatile fatty acid

(TVFA's) by using steam methods [61]. Microbial counts (bacteria and protozoa) in the ruminal fluid were determined using a counting cell (Hawksley, UK) as described by Demeyer [17]. Rumens volume was determined by the colorimetric method using Cr-EDTA according to El-Shazly [22]. Synthesized microbial protein (MP g/day) in the rumen was calculated according to Borhami [13].

Nylon bags technique was used to determine degradability kinetics of DM and CP. Two polyester bags (7x15 cm) with pore size of 45 μm were used for each incubation time. Approximately 5 g of air-dried diet (ground to 2 mm) were placed in each bag. All bags were incubated in the rumen, then they were withdrawn after 3, 6, 12, 24, 48 and 72 h; rinsed in tap water until the water became clear, then they were squeezed gently. Microorganisms attached to the residual sample were eliminated by freezing at -20°C [32]. Zero-time washing losses (a) were determined by washing 2 bags in running water for 15 min. The degradation kinetics of DM and CP were estimated (in each bag) by fitting the disappearance values to the equation $p=a + b(1-e)$ as proposed by McDonald [35]. The effective degradability (ED) for tested rations were estimated from the equation of McDonald [35], $ED = a + bc/(c + k)$, where ED (effective degradability), a (rapidly degraded fraction), b (slowly degraded fraction), c (rate of degradation) and k is the out flow rate assumed to be 0.05/h for concentrate under the feeding condition in this study.

Blood samples were collected at the end of the experimental period from all lambs. Blood samples were obtained from the jugular vein of the lambs in the morning before access to feed and water. Serum was obtained by centrifugation of blood and stored at -20°C until the analysis time. Glucose concentration was determined according to Trinder [54]; serum cholesterol [53]; serum total protein (TP) according to Henry [29], albumin (A) concentration according to Doumas [20]. Kidney function was evaluated by measuring blood urea using the colorimetric methods of Henry and Todd [30]. Liver function was

assessed by measuring the activities of aspartate aminotransferase (AST) and alanine amino transferase (ALT) by the method of Reitman and Frankel [48].

Antioxidant enzyme activities

The concentration of SOD was determined as described by Ogbunugafor [43]. The catalase activity (CAT) was determined as described by Usuh *et al.*, (2005) [57]. The activity of glutathione peroxidase (GPx) and reduced glutathione were determined by the method of Beutler [10]. Lipid peroxide concentration measured as thiobarbituric acid reactive substances (TBARS) according to Trota [56].

Statistical Analysis

The obtained data were subjected to statistical analysis using general linear models (GLM) procedure of SAS [49]. Significant differences among means were separated using LSD test according to Duncan [21] and significance was declared at $P<0.05$.

RESULTS AND DISCUSSIONS

Digestibility coefficients

All values of digestibility coefficient were significantly increased ($P<0.05$) for sheep supplemented with 50 gm carob pods compared to other groups (Table 2). Insignificant differences were found between the 25 and 50gm groups, while less ($p<0.05$) values were shown by 100 gm group. These could mainly be related to the effect of tannins in carob pods on DM intake whereas less DM intake could be found. Waghorn reported that condensed tannins could reduce fiber, CP and OM digestibility due to their binding properties and inhibition of rumen microbes and also they are ruminally indigestible [60]. Consequently, digestibility of dietary components will be affected and alter the end products of fermentation [37]. In this study digestibility of CP and CF was increased with the supplementation of carob pods (50 g/h/d). However, the action of tannins on animals probably depends on their solubility, in the gastrointestinal tract [9]. But this effect was in a certain level, as [52] and [59] indicating no adverse effect of dietary inclusion of CT below 5% level on nutrient intake and utilization. This was confirmed by the finding

in this study, for group fed 50 g/h/d. Group supplemented with 50gm carob pods had the highest ($P<0.05$) total digestible nutrients (TDN) and digestible crude protein (DCP) values than other groups. On the other hand

100 gm s0upplement was the less ($P<0.05$) value of TDN and DCP (Table 2). Other groups had intermediate values with insignificant effect ($P>0.05$). These results were agreed with the finding of Dey [18].

Table 2. Apparent digestibility coefficients, nutritive values and nitrogen utilization of the experimental diets

Items	Control	Carob pods			SEM	P value
		25	50	100		
DM intake (g/h/d):						
CFM	668.63	673.97	698.05	625.83	74.98	0.684
RS	363.79 ^{ab}	373.55 ^a	377.10 ^a	354.03 ^b	10.44	0.033
Total DMI, g	1032.42 ^a	1047.52 ^a	1075.15 ^a	979.86 ^b	44.14	0.042
Digestibility coefficients (%):						
DM	60.16 ^b	60.59 ^b	63.25 ^a	56.68 ^c	0.65	0.008
OM	62.96 ^b	63.36 ^b	65.84 ^a	59.72 ^c	0.49	0.001
CP	61.68 ^b	62.17 ^b	65.14 ^a	58.23 ^c	0.66	0.001
CF	57.09 ^b	57.88 ^b	60.47 ^a	54.04 ^c	0.98	0.006
EE	70.88 ^b	71.09 ^b	73.17 ^a	66.08 ^c	0.54	0.021
NFE	64.62 ^b	64.94 ^b	67.26 ^a	61.44 ^c	0.48	0.037
Nutritive values (%):						
TDN	60.60 ^b	60.96 ^b	63.34 ^a	57.41 ^c	0.50	0.018
DCP	6.34 ^b	6.37 ^b	6.71 ^a	5.93 ^c	0.11	0.021
Nitrogen utilization (g/h/d):						
N-intake (g/d)	16.99 ^a	17.16 ^a	17.72 ^a	15.98 ^b	0.87	0.031
Urine-N (g/d)	5.81 ^a	5.64 ^b	5.44 ^b	5.94 ^a	0.33	0.028
Fecal-N (g/d)	6.51 ^b	6.49 ^b	6.18 ^c	6.68 ^a	0.04	0.037
N-digested (g/d)	10.48 ^b	10.67 ^b	11.54 ^a	9.31 ^c	0.25	0.001
N-balance (g/d)	4.67 ^b	5.03 ^b	6.11 ^a	3.36 ^c	0.44	0.042
N- balance as % of N-digested	44.61 ^b	47.18 ^b	52.91 ^a	36.17 ^c	3.15	0.022

^{abc} means in the same row with different superscripts are significantly differ ($P<0.05$).

N-intake was lower ($P<0.05$) for group daily received 100 gm/h, while other groups had insignificant differences. Although all groups of animals had positive N balance, those receive 100 gm/h/d was the less ($P<0.05$) one. So, it was reflected the worst utilization one.

Source: Own results.

It is clear that the group received 50 gm carob pods group was the better ($P<0.05$) one in their N utilization compared with all groups. The less N utilization of that group daily received 100 gm/d, could be related to the tannin effect on reducing digestibility of dietary proteins by their ability to form insoluble complexes with proteins or digestive enzymes [10]. However, with increasing level of carob pods, NI decrease and both FN and UN increase, which resulted in less N utilization. So, the more ($P<0.05$) CP digestibility and N-utilization of rams daily supplemented with 50 gm./h, was within the level suggested by Bhatta et al. (2000) [11] and Dey et al. (2008) [19] as it appeared from the increase number of bacteria in the rumen as well.

Ruminal fermentation

Ruminal pH values were not significantly affected by different levels of carob pods supplementation (Table 3). So, no any phenomenon of acidosis was noticed, as carob pods have a potentiality to prevent ruminal acidosis by reducing rapid starch hydrolysis. Concentration of ruminal metabolites ($\text{NH}_3\text{-N}$ and VFA's) was significantly ($P<0.05$) varied among the experimental rations. Supplementation with 100 mg carob pods had the lower $\text{NH}_3\text{-N}$ and TVFA's concentrations; while 50 mg was recorded the highest value of TVFA's compared with other groups. However, tannins had limited effects on the values of pH, VFA production but resulted in a noticeable decrease in NH_3 . Molar proportion (%) of propionic (P) acid was not

significantly affected by different levels of carob pods supplementation, while acetic (A) acid, A:P ratio and rumen volume (L) were higher ($P<0.05$) with 50 mg supplementation. In the same mane, Beauchemin reported that increasing supplementation levels of quebracho CT resulted in decreasing total VFA concentration, but in contras acetate and A:P ratio were also reduced [6]. On the other hand, Carulla observed no change in total VFA concentration in sheep supplemented with black wattle tree CT, but acetate was decreased, while propionate increased [14]. Benchaar and Aguerre found that total concentrations of VFA and molar proportions of individual VFA were not affected by feeding quebracho CT [7] [1]. So, the effect of carob pods on total VFA concentration and VFA pattern have been variable among studies depending on the dosage rate and the consists of condensed tannin in carob pods

source [38], [24] and [41]. Microbial nitrogen synthesis (MN) was ranged from 14.95 to 17.51 (g/d) for 100 and 50 mg supplementation respectively, which reflected by the rate of out flow, as it considered as suitable for efficient ruminal cellulolytic bacteria and MN synthesis with the dose of 50 mg in this study. However, ruminal NH_3 - N levels for sheep fed 50 gm carob pods in this study, were higher than the critical level (25mg) suggested by Satter & Slyter [51] for microbial protein synthesis. Supplementation of carob pods significantly ($P<0.05$) decreased population of total Protozoa as compared with control. So, these results could end out that carob pods could change population of rumen microorganism. However, ruminal protozoa count is variable due to the tannins effects as it had been reported in many assays carried out in vivo [46].

Table 3. Effect of different levels of carob pods on rumen parameters of Barki sheep

Items	Control	Carob pods			SEM	P value
		25	50	100		
pH	6.35	6.41	6.49	6.47	0.21	0.639
NH_3 -N concentration (mg/100mlR.L)	15.16 a	14.88 ab	14.66 b	13.76 c	0.53	0.001
TVFA concentration (meq/100 mlR.L)	10.51 b	10.98 b	11.77 a	9.76 c	0.49	0.002
Acetic acid, %	54.88 b	55.17 b	56.74 a	52.54 c	0.58	0.001
propionic acid, %	23.73	23.89	23.97	23.15	1.12	0.659
Butyric acid, %	11.65 a	11.72 a	11.85 a	11.04 b	0.24	0.004
Acetic : propionic ratio	2.31 a	2.31 a	2.37 a	2.27 b	0.08	0.022
Rumen volume (L)	3.11 c	3.32 b	3.45 a	3.00 d	0.07	<0.0001
Rate of out flow (%h)	6.04 b	5.97 c	5.58 d	6.24 a	0.03	0.0001
Microbial N yield (g /d).	16.19 c	16.64 b	17.51 a	14.95 d	0.17	0.0001
Total bacteria counts, $\times 10^8$ cfu/ml	1.21 b	1.24 b	1.31 a	1.08 c	0.04	<0.0001
Total protozoa counts, $\times 10^6$ cfu /ml	4.53a	3.83 b	3.71 b	3.40 c	0.15	0.001

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

Source: Own results.

Degradation kinetics

It was illustrated in (Table 4) that washing loss fraction “a” for DM, and CP among groups was insignificantly different ($P> 0.05$) among groups. while, degradable fraction “b” and rate of degradation “c” was higher ($P<0.05$) for the control group than other groups. The effective degradability “ED” of DM or CP was lower ($P<0.05$) for group supplemented with 100 gm. carob pods.

While, it was higher for control group. These could be related to the less nutrients digestibility of 100 gm carob pods group, and as tannins are capable in binding with dietary proteins, resulted in less degradable nutrients in the rumen [9], [25], [45] and [40]. Although, CT could decrease ruminal degradability of CP, it increases amount of CP reaches the abomasums and small intestine [40].This could be confirmed in the present

study as RUP was less ($P < 0.05$) for group with 100 mg. carob pods.

Table 4. Degradation kinetics of DM and CP in the rumen of sheep fed the experimental diets

Items	Control	Carob pods			SEM	P value
		25	50	100		
DM						
A	23.25	23.28	23.31	23.30	0.38	0.638
B	52.84 ^a	50.42 ^b	49.78 ^b	45.63 ^c	0.76	0.003
C	0.043 ^a	0.040 ^b	0.039 ^b	0.036 ^c	0.01	0.0001
EDDM	46.57 ^a	46.32 ^a	46.40 ^a	44.97 ^b	0.44	0.004
CP						
A	21.53	21.46	21.40	21.31	0.29	0.589
B	57.77 ^a	55.98 ^b	54.11 ^b	51.88 ^c	0.24	0.004
C	0.076 ^a	0.066 ^b	0.064 ^b	0.056 ^c	0.004	0.0001
EDCP	56.85 ^a	52.67 ^b	51.38 ^b	48.44 ^c	1.36	0.001
RUP	43.15 ^c	47.33 ^b	48.62 ^b	51.56 ^a	1.41	0.002

^{a, b and c}: means in the same row with different superscripts are significantly ($P < 0.05$) different.

ED: effective degradability = $a + [bc/c + k]$, where k is passage rate (assumed to be 0.05/hr.).

RUP = 100 - ED (Orskov and McDonald, 1979).

a = soluble fraction (%). b = potentially degradable fraction (%).

c = rate of degradability (% h⁻¹). RUP = ruminally undegradable protein

Source: Own results.

Growth performance

Highest ($P < 0.05$) final body weight and average daily weight gain were recorded with lambs daily fed 50 gm carob pods, while the lowest values were recorded with the group fed 100 gm. Other groups had intermediate values without significant differences ($P > 0.05$). These could be due to the more feed intake of 50 gm carob pods followed by other groups and the less intake of 100 gm group.

Although that dietary CT generally tend to decrease DMI when it is in high level, but there are exceptions [42]; [47], the first two reported that no adverse effect on DMI, BW or ADG, while the second found that there is an increase in DMI. So, the last could be supported our finding with 50 gm supplementation of carob pods. However, this was reflected in more economic efficiency with 50 gm carob pods group.

Table 5. Growth performance of lambs fed experimental diets

Items	Control	Carob Pods			SEM	P value
		25	50	100		
Initial body weight, kg	24.64	24.58	24.39	24.61	1.36	0.7440
Final body weight, kg	37.45 ^b	38.03 ^b	40.65 ^a	34.26 ^c	1.08	0.0001
Daily weight gain, g/d	142.33 ^b	149.44 ^b	180.67 ^a	107.22 ^c	7.44	0.0001
Daily feed intake, g/d	1027.64 ^a	1033.16 ^a	1045.66 ^a	989.73 ^b	25.21	0.0172
Feed conversion ratio	7.22 ^b	6.91 ^b	5.79 ^c	9.23 ^a	0.46	0.0040
Economic efficiency:						
Average daily feed cost (L.E)	2.090 ^b	2.150 ^b	2.235 ^a	2.250 ^a	76.29	0.022
Price of daily gain(L.E)	5.27 ^b	5.53 ^b	6.68 ^a	3.97 ^c	0.3T2	0.005
Economical return (L.E /h/d)	3.18 ^b	3.38 ^b	4.45 ^b	1.72 ^a	0.21	0.029
Economic efficiency (%)	2.52 ^b	2.57 ^b	2.99 ^a	1.76 ^c	0.26	0.021
Relative economic efficiency	100.00 ^b	101.98 ^b	118.65 ^a	69.84 ^c	0.16	0.004

a,b,c different letters within a row denote significant differences between treatments ($P < 0.05$).

Source: Own results.

These results agreed with the finding of Alonso-Diaz who reported decline in body weight gain of goats with increasing concentrations of CT in their feed. He added that concentrations of CT beyond 6% tended to relate negatively on body weight gain

which supports with the observations of [2]. Lizardo and Andres-Elias reported that dietary carob (contained CT) did not affect the growth of weaned piglets [36] and [3]. However, many mammals, especially browsers, are able to produce proline-rich

salivary proteins (PRP) that are able to bind to dietary CT to inactivate them [5].

Blood parameters

The blood parameters are presented in Table 6. Daily supplementation of carob pods (100 gm) was resulted in less (P<0.05) blood glucose, total protein (TP) and urea-N, while other carob pods groups was shown more (P<0.05) concentration of TP and globulin, this could be due to the feed intake and digestibility coefficients for these groups.

On the other hand, control had the more concentration of glucose and cholesterol. Higher (P<0.05) concentration of albumin and urea-N was found for control and that supplemented with the less level of carob pods (25 and 50 gm/h/d).

However, all groups had insignificant different for liver and kidney functions (AST, ALT and creatinine). So, nothing unexpected phenomena happened in the present study.

Table 6. Blood serum biochemical components of lambs fed experimental diets

Items	Control	Carob pods			SEM	P value
		25	50	100		
Glucose (mmol/L)	1.16 ^a	1.08 ^{ab}	0.99 ^b	0.83 ^c	0.09	0.001
Cholesterol (mmol/L)	1.87 ^a	1.62 ^b	1.51 ^b	1.46 ^b	0.18	0.003
Total protein (g/L)	65.89 ^b	66.79 ^a	67.26 ^a	58.37 ^c	0.51	0.001
Albumin (g/L)	31.99 ^a	32.11 ^a	32.19 ^a	28.86 ^b	0.66	0.002
Globulin (g/L)	33.90 ^b	34.68 ^a	35.07 ^a	29.51 ^c	0.52	0.004
Urea N (mmol/L)	4.76 ^a	4.67 ^a	4.55 ^a	3.97 ^b	0.21	0.001
Creatinine (µmol/L)	96.77	96.89	97.11	97.44	0.88	0.647
AST (IU/L)	55.87	55.94	56.06	56.23	0.97	0.852
ALT (IU/L)	12.21	12.07	12.17	12.15	0.27	0.775

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

Source: Own results.

Antioxidant enzymes activity

Data in Table (7) indicated that supplementation with different levels of carob pods significantly (P<0.05) increased SOD, CAT, GPx and GST activities and decreased TBARS in plasma compared to the control group.

This revealed that carob pods could prevent the lipid peroxidation by enhancing the SOD, CAT and GPx activities. So, the phenolic compounds in carob exert powerful

antioxidant effects and inhibit lipid peroxidation by antioxidative enzymes [35], [34] and [28].

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 [27].

Table 7. Changes in the plasma antioxidant activities in lambs supplemented with different levels of carob (*Ceratonia siliqua L*)

Items	Control	Carob pods			SEM	P value
		25	50	100		
TBARS	0.41 ^a	0.29 ^b	0.25 ^b	0.22 ^b	0.07	0.004
GPx	12.63 ^b	14.87 ^a	15.88 ^a	15.52 ^a	1.38	0.001
GST	1.33 ^b	1.52 ^a	1.57 ^a	1.66 ^a	0.16	0.001
CAT	50.68 ^b	54.76 ^a	56.89 ^a	57.48 ^a	2.88	0.002
SOD	2.59 ^b	3.67 ^a	3.86 ^a	4.11 ^a	0.58	0.001

^{a and b:} means in the same row with different superscripts are significantly (P<0.05) different.

Source: Own results.

CONCLUSIONS

From this study it can be concluded that daily supplement with Carob Pods especially at 50

gm could improve the productive performances of the growing Lambs and achieved better feed efficiency, economic efficiency and relative economic efficiency.

Moreover, the hematological and biochemical parameters investigated in this study did not reveal any signs of illness in lambs due to daily consumption of 50 gm Carob Pods. However it needs more investigation for long term feeding in the field of beef or dairy milk production.

REFERENCES

- [1] Aguerre, M. J., Wattiaux, M. A., Capozzolo, M. C., Lencioni, P., Cabral, C., 2010, Effect of quebracho-chestnut tannin extracts at two dietary crude protein levels on performance and rumen fermentation of dairy cows. *J. Dairy Sci.* 93(Suppl. 1):445.
- [2] Alonso-Díaz, M.A., Torres-Acosta, J.F.J., Sandoval-Castro, C.A., Hoste, H., 2010, Tannins in tropical tree fodders fed to small ruminants: A friendly foe? *Small Ruminant Research* 89: 164-173.
- [3] Andres-Elias, N., Pujols, J., Badiola, I., Torrallardona, D., 2007, Effect of nucleotides and carob pulp on gut health and performance of weanling piglets. *Livest. Sci.* 108:280-283.
- [4] AOAC, 2007, Official Method of Analysis. (18th ed), Association of Official Analytical Chemists. Washington, DC, USA.
- [5] Austin, P.J., Suchar, L.A., Robbins, C.T., Hagerman, A.E., 1989, Tannins-binding proteins in saliva of deer and their absence in saliva of sheep and cattle. *J. Chem. Ecol.* 15, 1335-1347.
- [6] Beauchemin, K. A., McGinn, S. M., Martinez, T. F., McAllister, T. A., 2007, Use of condensed tannin extract from quebracho trees to reduce methane emissions from cattle. *J. Anim. Sci.* 85:1990–1996.
- [7] Benchaar, C., McAllister, T. A., Chouinard, P. Y., 2008, Digestion, ruminal fermentation, ciliate protozoal populations, and milk production from dairy cows fed cinnamaldehyde, quebracho condensed tannin, or *Yucca schidigera* saponin extract. *J. Dairy Sci.* 91:4765–4777.
- [8] Bengaly, S., Mhlongo, S., Nsahlai, I. V., 2007, The effect of wattle tannin on intake digestibility, nitrogen retention and growth performance of goats in South Africa. *Livestock Research for Rural Development* 19(4): Article 50.
- [9] Ben-Salem, H., Nefzaoui, A., Ben-Salem, L., Tisserand, J.L., 1999, Different means of administering polyethylene glycol to sheep: effect on the nutritive value of *Acacia cyanophylla* Lindl. Foliage *Anim. Sci.* 68:809–818.
- [10] Beutler, E., Dixon, O., Kelly, B.M., 1963, Improved method for the determination of blood glutathione. *J. Lab. Clin. Med.* 61: 882-890.
- [11] Bhatta, R., Krishnamoorthy, U., Mohammed, F., 2000, Effect of feeding tamarind (*Tamarindus indica*) seed husk as a source of tannin on dry matter intake, digestibility of nutrients and production performance of crossbred dairy cows in mid-lactation. *Anim. Feed Sci. Technol.* 83:67–74.
- [12] Biagi, G., Cipollini, I., Paulicks, B.R., Roth, F.X., 2010, Effect of tannins on growth performance and intestinal ecosystem in weaned piglets. *Arch. Anim. Nutr.* 64:121.
- [13] Borhami, B.E.A., Fahmy, W.G., El-Shazly, K., 1992, Rumen environment microbial protein synthesis and nitrogen balance in sheep. In: A Proceeding of "Manipulation of rumen micro-organisms". Inter. Confe. From. 20-23 Sept. 1992.
- [14] Carulla, J. E., Kreuzer, M. Machmüller, A., Hess, H. D., 2005, Supplementation of *Acacia mearnsii* tannins decreases methanogenesis and urinary nitrogen in forage-fed sheep. *Aust. J. Agric. Res.* 56:961–970.
- [15] Cheeke, P. R., Patton, N. M., Tempelton, G. S., 1982, Rabbit production. 5th Edition, Interstate Printers and Publishers, Danville 11.
- [16] Custodio, L., Fernandes, E., Escapa, A. L., Fajardo, A., Aligue, R., Albericio, F., Neng, N. R., Nogueira, J. M. F., Romano, A., 2011, Antioxidant and cytotoxic activities of carob tree fruit pulps are strongly influenced by gender and cultivar. *J. Agric. Food Chem.* 59:7005-7012
- [17] Demeyer, I., 1981, Rumen microbes and digestion of plant cell walls. *Agriculture and Environment*, 6, 295-337.
- [18] Dey, A., Dutta, N., Sharma, K., Pattanaik, A. K., 2007, *Livestock Research Rural Development*. 19 (12): 295.
- [19] Dey, A., Dutta, N., Sharma, K., Pattanaik, A.K., 2008, Effect of dietary inclusion of *Ficus* performance of lambs. *Small Rum Res.*, 75:105–114.
- [20] Doumas, B.T., Watson, W.A., Biggs, H.G., 1971, Albumin standards and measurement of serum with bromocresol green. *Clin. Chem. Acta.*, 31(1): 87-96.
- [21] Duncan, D.B., 1955, Multiple ranges and multiple F- test. *Biometric* 11: 1-42.
- [22] El-Shazly, K. Ahmed, E.I.A., Naga, M.A., Borhami, B.E.A., 1976, A calorimetric technique using chromium-ethylen diamins tetracetate for measuring rumen volume. *J. Agric. Sci. Camb.*, 87: 369.
- [23] FAO. 2009. FAOSTAT statistical database. Rome (available at faostat.fao.org).
- [24] Frutos, P., Hervás, G., Giráldez, F.J., Fernández, M., Mantecón, A.R., 2000, Digestive utilization of quebracho-treated soya bean meal in sheep. *J. Agr. Sci.*, 134, 101-108.
- [25] Frutos, P., Hervás, G., Giráldez, F.J., Mantecón, A.R., 2004, Review. Tannins and ruminant nutrition. *Spanish Journal of Agricultural Research* 2: 191-202.
- [26] Gasmi-Boubaker, A., Bergaoui, R. Khaldi, A. Mosquera-Losada, M. R., Ketata, A., 2008, First attempt to study carob pulp utilization in rabbits feeding. *World J. Agric. Sci.* 4(1):67-70.
- [27] Goñi, I., Serrano, J., 2005, The intake of dietary fiber from grape seeds modifies the antioxidant status in rat cecum. *J. Sci. Food Agric.*, 85: 1877-1881.
- [28] Harborne, J.B., Baxter, H., 1999, *The Handbook of Natural Flavonoids*, Vol 2. John Wiley and Sons, New York.
- [29] Henry, R.J., Cannon, D.C., Winkelman, J.W., 1971, *Clinical chemistry: Principles and Techniques*.

- (11th ed.), Happer and Row Publishers, New York, USA, pp. 1629.
- [30] Henry, J.B., Todd, S.D., 1974, Clinical Diagnosis and Measurement by Laboratory Methods. (16th ed.), WB Saunders and Co. Philadelphia, PA, USA, pp. 260.
- [31] Jansman, A. J. M., Enting, H., Verstegen, M. W. A., Huisman J., 1994, Effects of condensed tannins in hulls of faba beans (*Vicia faba* L.) on the activity of trypsin and chymotrypsin in digesta collected from the small intestine of pigs. *Brit. J. Nutr.*, 71:627-641.
- [32] Kamel, H. Sekine, J. Suga, T., Morita, Z., 1995, The effect of frozen-rethawing technique on detaching firmly associated bacteria from in situ residues. *Can. J. Anim. Sci.*, 75:481.
- [33] Karabulut, A., Canbolat, O., Kamalak, A., 2006, Evaluation of carob, *Ceratonia siliqua* pods as a feed for sheep. *Livest. Res. Rural Dev.* 18(7): 277.
- [34] Klenow, S., Jahns, F., Pool-Zobel, L., Gleis, M., 2009, Does an extract of carob (*Ceratonia siliqua* L.) have chemopreventive potential related to oxidative stress and drug metabolism in human colon cells. *J. Agriculture and Food Chemistry* 57; 2999–3004.
- [35] Kumazawa, S., Taniguchi, M., Suzuki, Y., Shimura, M., Kwon, M., Nakayama, T., 2002, Antioxidant activity of polyphenols in Carob pods. *J. Agric. Food Chem.* 2002; 50: 373-377.
- [36] Lizardo, R., Canellas, J., Mas, F., Torrallardona, D., Brufau, J., 2002, Utilization of carob powder in piglet diets and its influence on growth performance and health after weaning. 34emes Journées de la Recherche Porcine, sous l'égide de l' Association Française de Zootechnie, Paris, France, 5-7 Fevrier.
- [37] Lowery, R.S., Bowman, M. C., Knox, F. E., 1969, Effect of Bidrin on the metabolism of dietary components by bovin. *J. Dairy Sci.*, 52:1463.
- [38] Makkar, H.P.S., 2003, Effects and fate of tannins in ruminant animals, adaptation to tannins, and strategies to overcome detrimental effects of feeding tannin-rich feeds. *Small Ruminant Research* 49: 241-257
- [39] McDonald, I. 1981, A revised model for the estimation of protein degradability in the rumen. *J. Agric. Sci. Camb.*, 96(1): 251-252.
- [40] Min, B.R., Barry, T.N., Attwood, G.T., McNabb, W.C., 2003, The effect of condensed tannins on the nutrition and health of ruminants fed fresh temperate forages: a review. *Anim. Feed Sci. Technol.*, 106:3–19.
- [41] Mueller-Harvey, I., 2006, Unravelling the conundrum of tannins in animal nutrition and health. *J. Sci. Food Agric.* 86(13):2010-2037.
- [42] NRC, 2007, Nutrient requirements of small ruminants: sheep, goats, cervids, and New World camelids. National Research Council of the National Academies, National Academies Press, Washington, DC, USA.
- [43] Ogbunugafor, H.A., Sofidiya, O., Okpuzor, J., Kemdilim, M., Anajekwe, B. and Ekechi, A., 2010, Effect of extracts of *Hymenocardia acida* Tul (*Hymenocarpaceae*) on rats. *Journal of American Science* 6: 143-146.
- [44] Ortiz, L. T., Rodriguez, M. L., Alzueta, C., Rebole, A., Centeno, C., Trevino, J., 2004, Effect of carob seed (*Ceratonia siliqua* L.) in broiler chick diets on nutrient digestibility and intestinal viscosity. In: EAAP Publication 110, Spain, pp. 239-242.
- [45] Ørskov, E.R., McDonald, I., 1979, The estimation of protein degradability in the rumen from incubation measurements weighed according to rate of passage. *J. Agric. Sci. Camb.*, 92(2): 499-503.
- [46] Preston, T.R., 1995, Biological and chemical analytical methods. In. Preston, T.R. (Ed.) Tropical animal feeding: a manual for research workers. Rome: FAO, P.191-264.
- [47] Priolo, A., Lanza, M., Biondi, L., Pappalardo, P., Young, O. A., 1998, Effect of partially replacing dietary barley with 20% carob pulp on post-weaning growth, and carcass and meat characteristics of Comisana lamb. *Meat Sci.* 50(3):355-363.
- [48] Reitman, S., Frankel, S., 1957, A calorimetric method for the determination of serum glutamic oxaloacetic and glutamic pyruvic transaminases. *Amer. J. Clin. Path.*, 28(1): 56-63.
- [49] SAS. 2009. SAS/STAT® 9.2 User's Guide. (2nd ed.), SAS Institute Inc, Cary, NC, USA.
- [50] Serrano, J., Puupponen-Pimiä, Dauer, A., Aura, A., Saura-Calixto, F., 2009, Tannins: Current knowledge of food, sources, intake, bioavailability and biological effects. *Mol. Nutr. Food Res.* 53:S310- S329.
- [51] Satter, L.D., Slyter, L.L., 1974, Effect of ammonia concentration on rumen microbial protein production in vitro. *Br. J. Nutr.*, 32: 199-208.
- [52] Terrill, T. H., Douglas, G. B., Foote, A. G., Purchas, R.W., Wilson, G. F., Barry, T.N. 1992, Effect of condensed tannins upon body growth, wool growth and rumen metabolism in sheep grazing sulla (*Hedysarum coronarium*) and perennial pasture. *Journal Agriculture Science Cambridge* 119: 265-273.
- [53] Tietz, N.W., 1986, Textbook of Clinical Chemistry. WB Saunders, Philadelphia, USA.
- [54] Trinder, P., 1969, Determination of blood glucose using an oxidase-peroxidase system with a non-carcinogenic chromogen. *J. Clin. Pathol.*, 22(2): 158-161.
- [55] Tous, J., Romero, A., Hermoso, J. F., Ninot, A., Plana, J., Batlle, I., 2009, Agronomic and commercial performance of four Spanish carob cultivars. *HortTechnology* 19:465-470.
- [56] Trotta, R. J., Sullivan, S. G., Stern, A., 1982, Lipid peroxidation and haemoglobin degradation in red blood cells exposed to t-butyl hydroperoxide. *Biochem. J.* 204, 405-415.
- [57] Usuh, F.I., Akpan, E.J., Etim, E.O., Farombi, E.O., 2005, Antioxidant actions of dried flower of *Hibiscus sabdariffa* L. on sodium arsenite induced oxidative stress. *Pak. J. Nutr.*, 4:135-141.
- [58] Van Soest, P.J., Robertson, J.B., Lewis, B.A., 1991, Methods for dietary fiber, neutral detergent fiber and non-starch polysaccharides in relation to animal nutrition. *J Dairy Sci.*, 74(10): 3583-3597.

[59]Waghorn, G.C., Shelton, I.D., McNabb, W.C., 1994, Effects of condensed tannins

in *Lotus pedunculatus* on its nutritive value for sheep.

1. Non-nitrogenous aspects. Journal Agriculture Science Cambridge 123, 99-107.

[60] Waghorn, G. C., 2008, Beneficial and detrimental effects of dietary condensed tannins for sustainable sheep and goat production-progress and challenges. Anim. Feed Sci. Technol. 147:116–139.

[61]Warner, A., 1964, Production of volatile fatty acids in the rumen, methods of measurement. Nutr. Abst. Rev., 34: 339-352.