EFFECT OF FEEDING OAK (*Quercus aegilops*) ACORNS ON MILK PRODUCTION, MILK COMPOSITION AND SOME BLOOD BIOCHEMICAL PARAMETERS OF BLACK GOATS

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ABSTRACT

Twenty four Black goat does at 1st week post kidding were adapted to control diet for 2 weeks and then blocked into 4 groups (6 does/group) to be fed on one of the experimental diets (0, 5, 10 and 15% oak acorns) to study the effect of different levels of oak acorns on milk yield, milk composition and some serum biochemical traits. Results revealed that at 10th week of lactation, there was a significant increase in daily milk yield in does fed on 15% oak acorns. In contrast the group fed on 15% oak acorns exhibited a significant decrease in milk protein and solid non-fat contents at 10th week of lactation. The milk lactose yield was significantly higher in group fed on 15% oak acorns at 10th week of lactation. The serum glucose concentration at 8th week of lactation was significantly higher in does fed on 10% oak acorns while at 10th week; it was significantly higher in does fed 5% oak acorns. It could be concluded that feeding does on diets containing 15% oak acorns caused a significant increase in daily milk yield of Black goats and a significant decrease in milk protein content.

تأثير تغذية البلوط الحلو (*Quercus aegilops*) على أنتاج وتركيب الحليب و بعض الخواص الكيموحيوية في الماعز المحلي

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المستخلص

تم تغذية أربعة و عشرين معزة في الأسبوع الأول بعد الولادة على عليقة السيطرة لمدة أسبوعين و بعد ذلك قسمت بصورة عشوائية الى أربع مجاميع (ستة معزة لكل مجموعة)لتتغذى على أحدى العلائق التجريبية (0%, 5%, 10%, 15% بلوط) لدراسة تأثيرتغذية مستويات مختلفة من البلوط على كمية و تركيب الحليب و بعض الصفات الكيموحيوية للدم في المعزات الحلوبة. أظهرت النتائج بوجود زيادة معنوية في الأنتاج اليومي للحليب و الأنتاج اليومي للاكتوز لدى االمجموعة المغذاة على العليقة المحتوية على 15% بلوط في الأسبوع العاشر من فترة الحلب بينما نسبة البروتين و المواد الصلبة غير الدهنية في الحليب كانت أقل معنويا . كانت هناك زيادة معنوية في مستوى الكلوكوز في مصل الدم للمجموعة المغذاة على العليقة التي أحتوت على 10% بلوط في الأسبوع الثامن من فترة الحلب بينما في الأسبوع العاشر كانت هناك زيادة معنوية لدى المجموعة المغذاة على عليقة 5% بلوط. يمكن الأستنتاج بأن أدخال البلوط الحلو في علائق الماعز يعمل على زيادة أنتاج الحليب اليومي كما يؤثر على نسبة البروتين في الحليب و مستوى السكر في الدم.

كلمات مفتاحية: عليقة، لاكتوز، البروتين، المواد الصلبة.

Received:10/2/2020, Accepted:12/5/2020

INTRODUCTION

It is known that a major restriction on livestock production in the world is the scarcity of the year-round feedstuffs supply. Thus to overcome this shortage, it might be achieved through better utilization of non-conventional feeds such as trees, shrubs, pods, fruits and seeds that do not compete with human food (2), high in protein and minerals and that can be used as supplements to improve the nutritional value of conventional poor quality roughages (18). Among those acorns from different oak species are considered very energetic alternative sources and have potential nutritive values for ruminant animals due to their high starch contents and may be used to some extent in sheep and goats diets where there is food scarcity (8). Oak acorns, however, have some adverse effects on animal production, which arise from the presence of some anti-nutritional factors such as tannins and phenolic compounds (19). Tannins are found throughout the oak plant, with higher levels found in the leaves, buds, twigs, and acorns (21). Acorns contain about 6-8 percent tannins. The tannins and their metabolites are absorbed from the animal's gastro-intestinal tract and enter the blood stream (5). The low intake of tannins by small ruminants, may lead to positive effects mainly on nitrogen metabolism (17; 24). Depending on the molecular structure, condensed tannins may improve the digestive utilization of feed by ruminants, mainly because of a reduction in protein degradation in rumen that is beneficial increasing the amino acid flow to the small intestine (13). Merkhan (14) reported a positive effect on milk yield and its components yield in goats by supplementing the diet with 14% oak acorns. Also, Buccioni (6) demonstrated a greater milk production by sheep following consumption of a diet that contained 8% chestnut tannin extract.

MATERIALS AND METHODS

This experiment was conducted at the Animals project of college of Agricultural Engineering Sciences, University of Duhok. Preparation of Plant Material Oak acorns were collected at Hadena village at Duhok governorate, Kurdistan region, Iraq and stored in shed at 25C˚ for 15 days, then grinded using a hammer mill and were analyzed in for DM, OM, ash, EE, CP, CF, condensed tannins, total phenolics and total flavonoids (Table 1), and later mixed with other feed ingredients in experimental diets. Determination of Condensed Tannins The condensed tannin content in the samples of oak acorns was determined according to Makkar (11). 10ml of 70% aqueous acetone was added to 0.2g of dried ground sample and in 4˚C centrifuged at 3000g for 10 minutes. 3ml of butanol-HCl reagent (butanol-HCI 95:5 v/v) and 0.1ml of Ferric reagent (2% of ammonium iron sulfate in 2N HCl) were added to 0.5ml of supernatant, vortexed and put in heating block at 97C° for one hour. After the tubes were cooled, the absorbance was red at 550nm via spectrophotometer (Jenway, UK) and condensed tannins were calculated as following: (A 550 nm x 78.26 x Dilution factor*) / (% of DM). Determination of Total Phenolics The total phenolics content of oak acorns was determined using Folin-Ciacolteu reagent method as shown by Makkar (11). The extract was prepared by adding 10ml of either methanol or distilled water to 200mg of dried ground oak acorn. Suitable aliquots of extract (0.02, 0.05 and 0.1 ml) were taken to test tubes. The volume was made up to 0.5ml via distilled water.0.25ml of Folin-Ciacolteu reagent and 1.25ml of 20% sodium carbonate solution were added. The mixture was allowed to stand for 45 minutes in darkness. The absorbance was read via spectrophotometer at 725nm (VersaMax molecular devices, USA). A calibration curve was obtained with 5 different concentrations of standard gallic acid solution in the range of 62.5-1000µg/ml. Determination of Total Flavonoids Determination of total flavonoids of oak acorn extracts was determined using Aluminum Chloridimetric method according to Woisky and Salatino (25). Quercetin was used as a standard flavonoid to determine flavonoid content. A calibration curve was obtained with 5 different concentrations of standard quercetin solution in the range of 4-12µg/ml. To 100 microliter of extract, 1ml of potassium acetate and 1 ml of 5% AlCl (wt. /v.) were added. The mixture was let to stand for 30 minutes, and then absorbance was read at

425nm via spectrophotometer (VersaMax molecular devices, USA).

Table 1. Composition of oak (*Quercus aegilopos*) acorn, all data based on g/kg dry matter

DM	OM	EE	CP	CF	NFE	ME	CT	T- Phenolics		T- Flavonoids	
								1	2	1	2
593.0	968.8	87.5	42.0	73.2	359.1	214.	3.76	125.08	94.61	9.06	14.06

DM: dry matter, OM: organic matter, EE: ether extract, CP: crude protein, CF: crude fiber, NFE: nitrogen free extract, ME: metabolizable energy, CT: condensed tannin, T: total, 1: water extraction, 2: methanol extraction.

Experimental Animals

Twenty-four lactating Black does with live weight of 32 ± 2Kg were purchased from a local farm at their first week post kidding. The does were fed on control diet for 14 days as an adaptation period, then were blocked based on their live weight and parity into 4 groups (6 does per treatment) and were housed individually (4m² /pen) indoor in during the 8 weeks period of study. Preparation of Experimental Diets The ingredients of the experimental diets are shown in Table 2. The samples of feedstuffs and diets were analyzed in triplicates according to the methods given by AOAC (1) for DM, OM, ash, EE, CP and CF as shown in Table 2. Based on laboratory analyses, 4 diets were formulated to represent T1 (0% oak acorns), T2 (5% oak acorns), T3 (10% oak acorns) andT4 (15% oak acorns). The diets were prepared according to AFRC (1) to produce 1Kg of milk per day. Experimental Procedure The does were kept with their kids in individual pens and fed on experimental diets twice daily at 8:30 a.m. and 4:30 p.m. Feed was weighed daily using a metric scale. Feed refusals were recorded twice weekly. Blood sampling and analysis The blood samples were collected at biweekly interval on week 0, 2, 4 and 6 of experimental period through jugular venipuncture and transferred into a silica containing tube. The blood samples were kept at 4C° overnight before being centrifuged for serum preparation (section). The serum was kept at -18C° for subsequent analysis for total protein, albumin, globulin, glucose and triglycerides using a blood analyzing machine (Cobas, Germany).

Table 2. Ingredients of the experimental diets and proximate analysis of all treatments

Feedstuffs %	T1	T2	T3	T4
Barley	50	45	40	40
Wheat bran	13	12	11	10
Soybean meal	15	16	17	18
Corn	10	10	10	5
Wheat straw	10	10	10	10
Oak acorns	0	5	10	15
Vitamins-minerals premix	1	1	1	1
Iodized Salt	1	1	1	1
DM (g/kg)	901	897	903	903
OM (g/kg DM)	939	935.5	937.5	940.8
Ash (g/kg DM)	61	64.5	62.5	59.2
CP (g/kg DM)	137	148	148	149
EE (g/kg DM)	32	29.9	23.3	28.5
CF (g/kg DM)	151	151	154	152
NFE[1] (g/kg DM)	480	496.4	484.8	485.7
ME[2] (MJ/kg DM)	11.2	11.6	11.2	11.42

DM: dry matter, OM: organic matter, EE: ether extract, CP: crude protein, CF: crude fiber, NFE: nitrogen free extract, ME: metabolizable energy, [1]NFE%= 100- (Water % + Ash % + EE % + CP % + CF %), [2]ME was calculated according to [15],ME= (CP*0.02+EE*0.031+Cf*0.005+NFE*0.014)

Milk yield and milk analysis

Milk yield was determined at week 4, 6, 8 and 10 post kidding. The yield was determined using double oxytocin injection method as shown by Doney (7). The does were injected with 1mL of oxytocin (Oxytocin, Netherlands) to stimulate milk let down and milked via hand milking. The goat kids were separated from their dams for 4 hours, and then the dams were re-injected with 1mL of oxytocin and milked again. The milk yield of 24 hours was calculated by multiplying the yield within 4 hours by 6. Milk samples (50mL) from each doe was kept at -18C$^{\circ}$ for subsequent analysis. Milk samples were analyzed for composition after being defrosted in fridge, using an ultrasonic milk analyzer (Ekomilk, USA). Statistical Analysis All measured parameters were statistically analyzed by an ANOVA procedure of Genstat (Genstat 17th edition, VSN, UK) as a complete randomized block design with repeated measurement (effect of time). Data of week 4 were used as covariates for the other weeks and analyzed as repeated measurement analysis by Fisher's least significant difference test.

RESULTS AND DISCUSSION

Milk Yield The effect of different dietary treatments of oak acorns on daily milk yield is presented in Table 3. Repeated measures analysis of variance showed neither the time nor the interaction between time and oak level in the diet had significant effect (P> 0.05) on daily milk yield. Also, there were no significant (P>0.05) effects of feeding oak acorns (different levels) on daily milk yield at the 6th and 8th week of lactation. However, at 8th week, does fed on T4 tended to produce higher (P=0.09) amount of milk (1042g/d) as compared to that of control (553g/d).

Table 3. Effect of feeding different levels of oak acorns (*Quercus aegilops*) on daily milk yield (g/d) of Black goats

Week	T1	T2	T3	T4	SED	P value
Week4	750	528	528	756	210.9	0.53
Week6	648	600	510	774	147.4	0.37
Week8	553	642	624	1042	194.7	0.09
Week10	468^{a}	649^{ab}	517^{a}	868^{b}	128.7	0.03

Different letters within the same raw refers to significant difference

At 10th week of lactation, does fed on T4 (15% oak acorns) were observed to produce a significantly (P=0.03) higher amount of daily milk (868g/d) as compared to that of control (468g/d) and T3 (517g/d). This result is in accordance with that of Merkhan (14) who found a significant increase in daily milk yield of goat does at mid-lactation fed on pasture and 0.5Kg barley/head/day supplemented with 14% oak acorns as compared to those of control. Similar results were reported by Alipanahi (3) who found no significant effects of feeding lactating goats on a diet consisting of 10% oak acorns and extruded soybean on efficiency of daily milk production. The contribution of tannins (particularly condensed tannins) may also have a bearing in overall nutrient utilization Sharma (18). In this study the condensed tannins from acorn may had affected positively on daily milk yield as explained by Singh and Bhat (20) that the condensed tannins are beneficial for enhancing animal production as a result of increased protein outflow from the rumen to abomasum and intestine and associated with a decreased urinary nitrogen excretion. Moreover, Min (15) demonstrated that the level of tannins within the range of 10-40 g/ kg DM, may improve the feed utilization and this may lead to greater absorption of available amino acids in the small intestine. In addition, Taha (22) found an increase in milk yield when he supplemented alfalfa silage with 25 g/kg DM chestnut tannin. Furthermore Lamy (9) reported that tannins react with the protein in the salivary prolin, the concentration of prolin is varying among animals depending on species, physiological state and geographical region. Prolin seems to be more active and concentrated in the animal that found in the tropical region such as goat and deer compared to cattle and sheep (16; 23). Milk Composition and Milk Components Yield The effect of feeding experimental diets on milk composition is presented in Table 4. The effect of dietary treatments on milk components yield is shown in Table 5. Repeated measures analysis of variance showed an effect of time on mean milk fat content (P= 0.003), milk protein content (P= 0.05) and daily fat yield (P= 0.006). There was no effect of interaction between time and level of acorns in the diet on milk components except for daily lactose yield (P= 0.04). Also, there were no significant (P>0.05) effects of dietary treatments on fat percentage and daily fat yield among the does milk throughout the experimental period. Alipanahi (3) reported that feeding lactating Kurdish goats on a diet consisting of 10% oak acorns had no effect on the concentrations of acetate and butyrate in rumen fluid and on plasma triglycerides and this may partly explain why milk fat content is unaffected by feeding oak acorns as demonstrated by Mansbridge and Blake (12) that milk fat is derived from *de novo* synthesis using circulating acetate and butyrate that originate from the rumen and uptake of plasma lipids . There were no significant (P>0.05) effect of dietary treatments on milk protein percentage in weeks 4, 6 and 8 of lactation, while at week 10, the milk from T4 does had significantly (P=0.05) lower amount of protein (4.48%) as compared to that of control (5.49%). This may be attributed partly to no effect of oak acorns on plasma protein concentrations (3), While the significant lower milk protein content at 10th week of lactation in the does which received T4 may be related to the significantly higher daily milk production by does fed on T4. The effect of treatments on daily protein yield is absent. Also, throughout the experimental period, inclusion of oak acorn levels had no significant (P>0.05) effect on milk lactose content. However, at 8th week of lactation, the does fed on T4 tended (0.09) to produce more milk lactose yield, whereas at 10th week of lactation, they exhibited a significantly increased daily milk lactose yield (38.44 g/d) as compared to that of control (22.99 g/d).

Table 4. Effect of feeding different levels of oak acorns on milk composition of Black goats.

Milk constituent *	Week	T1	T2	T3	T4	SED	P value
Fat	4	3.06	2.8	3.82	3.17	0.818	0.64
	6	2.64	3.17	3.9	2.92	0.53	0.14
	8	4.68	3.55	4.43	3.88	0.589	0.24
	10	4.34	3.72	5.07	3.16	0.888	0.20
Protein	4	4.5	4.41	4.12	4.51	0.688	0.93
	6	5.09	4.43	4.39	4.53	0.453	0.39
	8	4.9	4.21	4.51	4.22	0.302	0.11
	10	5.49^{b}	4.92^{ab}	4.73^{ab}	4.48^{a}	0.336	0.05
Lactose	4	4.47	4.45	4.38	4.44	0.070	0.60
	6	4.51	4.44	4.41	4.45	0.054	0.29
	8	4.45	4.41	4.41	4.39	0.035	0.48
	10	4.51	4.47	4.42	4.44	0.041	0.15
SNF	4	10.16	9.61	9.21	9.7	0.818	0.71
	6	10.44	9.62	9.56	9.73	0.551	0.38
	8	10.14	9.34	9.69	9.33	0.366	0.12
	10	10.85^{b}	10.19^{ab}	9.91^{ab}	9.67^{a}	0.404	0.05

*: g/100 g milk, Different letters within the same raw refers to significant difference

Table 5. Effect of dietary levels of oak acorns on daily milk components yield in Black goats

Component yield*	Week	T1	T2	T3	T4	SED	P-value
Fat	4	22.52	11.42	19.89	24.03	7.87	0.41
	6	18.65	18.6	19.76	21.21	4.95	0.94
	8	25.3	22.07	26.6	39.86	7.24	0.11
	10	21.3	24.14	23.02	28.16	5.07	0.58
Protein	4	32.24	17.24	21.4	22.2	8.23	0.18
	6	32.09	26.99	22.52	34.67	7.04	0.34
	8	26.85	26.84	27.62	43.72	8.36	0.15
	10	27.64	31.93	23.75	38.27	5.04	0.06
Lactose	4	33.54	17.74	23.12	33.5	8.53	0.21
	6	29.1	26.67	22.5	34.47	6.57	0.35
	8	24.56	28.28	27.47	45.75	8.6	0.09
	10	22.99^{a}	29.03^{ab}	22.84^{a}	38.44^{b}	5.64	0.04
SNF	4	69.89	37.93	48.25	72.3	17.43	0.18
	6	66.58	58.21	48.9	74.94	14.69	0.35
	8	55.77	59.71	59.85	97	18.3	0.12
	10	54.91^{ab}	66.13^{ab}	50.51^{a}	83.17^{b}	11.44	0.05

*: g/kg, SNF: solid non-fat

Furthermore, the dietary treatments also had no effect (P>0.05) on milk solid non-fat content and daily solid non-fat yield except at 10th week of lactation, in which the does that consumed T4 had a significantly (P=0.05) lower milk solid non-fat percentage (9.67 %) as compared to that of control group (10.85 %).Serum Biochemical Parameters Repeated measures of analysis showed no effect of time on serum biochemical properties of lactating does as fed on oak acorns. Table 6 represents effect of feeding oak acorns on some serum biochemical properties. There were no significant (P>0.05) effects of oak acorns inclusion on serum protein in lactating does.

Table 6. Effect of feeding dietary levels of oak acorns on serum biochemical parameters in lactating Black goats

Parameter (mg/dL)	week	T1	T2	T3	T4	SED	P value
Total protein	4	6.60[a]	8.15[b]	6.80[a]	6.30[a]	0.29	<.001
	6	7.55	7.50	7.42	7.00	0.60	0.28
	8	6.80	7.35	7.77	7.05	0.49	0.43
	10	6.88	7.42	7.45	7.2	0.50	0.65
Albumin	4	3.05	2.70	2.87	2.85	0.21	0.47
	6	3.12	2.70	2.82	3.27	0.23	0.10
	8	3.22	2.87	2.97	3.15	0.21	0.37
	10	3.07	3.07	2.92	3.27	0.24	0.57
Globulin	4	3.55[ab]	5.45[c]	3.92[b]	3.45[a]	0.20	<.001
	6	4.42	4.80	4.60	3.73	0.59	0.34
	8	3.60	4.50	4.80	3.90	0.26	0.44
	10	3.80	4.35	4.53	3.92	0.535	0.50
Glucose	4	73.0	66.20	56.2	64.5	6.9	0.169
	6	65.0	79.70	72.5	54.2	10.91	0.18
	8	45.20[a]	47.8[ab]	64.50[b]	49.2[ab]	6.81	0.05
	10	44.50[a]	57.8[b]	49[ab]	44.2[a]	4.47	0.03
Triglyceride	4	14.80	20.20	15.20	15.50	4.12	0.53
	6	17.20	18.0	16.20	13.80	3.22	0.59
	8	14.20	12.20	11.2.0	13.0	3.12	0.80
	10	14.0	18.0	16.25	12.75	2.78	0.28

Also does serum albumin, globulin and triglycerides means were not affected (P>0.05) by the dietary treatments. At 8th week post parturition, a significant (P=0.05) increase was noted in serums glucose level from does fed on T3 as compared to that of control (64.5 vs. 45.2 mg/dL). While at 10th weeks post parturition, does fed on T3 showed a significantly (P=0.03) higher serum glucose level (57.8 mg/dL) as compared to that of control T4 (44.5 mg/dL) and (44.2 mg/dL) respectively. The results are comparable to that of Alipanahi (3) who found no effect of feeding a diet containing extruded soybean and 10% oak acorns on plasma protein, albumin, glucose, triglyceride in multiparous lactating Kurdish goats within 6 weeks of lactation period. Similarly, Taha (23) also did not find any changes of blood serum biochemical parameters when he fed lactating ewes alfalfa silage supplemented with different levels (25, 50 or 75 g/kg DM) of chestnut hydrolysable tannin. In conclusion, feeding oak acorns to lactating goats improved daily milk yield and daily milk lactose yield and affected milk protein content and serum glucose concentration. Feeding oak acorns had no deleterious effects on goat's health. Further work is needed to explain the positive effect of oaks on milk yield and studying its effect on lactation persistency.

ACKNOWLEDGMENT

The authors wish to thank Professor Dr. Jalal E. Alkass for his help in providing valuable scientific consultancy

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