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EFFECT OF FEEDING OAK (*Quercus aegilops*) ACORNS ON NUTRIENT DIGESTIBILITY, NITROGEN BALANCE, RUMINAL FLUID CHARACTERISTICS AND SOME BLOOD METABOLITES IN SHEEP AND GOATS

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ABSTRACT

This study aimed to examine the response of sheep and goats to dietary effects of feeding different levels of oak (*Quercus aegilops*) acorns in terms of nutrient digestibility, nitrogen balance, rumen function and some serum metabolites. The study was conducted over 4 periods each of 15 days; in each period, rams and bucks were allocated to one of the dietary treatments (control, 5, 10 and 15% oak acorns). Rams and bucks exhibited different responses to the effects of dietary levels of oak acorns. The digestibility coefficient of DM was reduced in both rams and bucks upon feeding acorns, but this decline was significant (P≤0.05) only in rams fed 10 and 15% acorns as compared to rams fed control. Also, the rams and bucks showed numerical decrease in digestibility coefficients of OM, CP, CF, and NPN. The experimental treatments had no effects on nitrogen balance parameters. Bucks exhibited significantly (P≤0.05) higher concentrations of rumen ammonia-nitrogen before, 4 hours and 6 hours after morning feeding. The rumen pH value was significantly (P≤0.05) by the animal species only at the time before morning feeding. The serum metabolites were not affected by the level of acorns in the diet. The results of this study indicate that goats are superior in digesting diets containing oak acorns up to 15%.

KEYWORDS: *Quercus aegilops*, Digestibility, Sheep, Goat.

INTRODUCTION

The intensive and efficient use of the biomass from the forages, crop residues including agro-industrial by-products and other non-conventional feed resources to the possible extent will be effective manners for the of enhancement of productivity and performance as efficient use of both of the feed resources and feed security play a major role in maximizing animals productivity (Devendra and Leng, 2011). Different alimentary strategies are used by the different animal species such making changes in voluntary intake, ingested food items, or modifying digestive physiology (Lamy et al., 2011). The same authors reported that grazing herbivores get their nutritional demands by giving priority to certain nutritional parameters by selecting quantities and types of different feeds, this is usually achieved by avoiding or regulating consumption of plant secondary compounds.(Lamy et al., 2011). Oak (Quercus spp.), a genus under the family Fagaceae, is an expanded group of hardwood trees. This species is distributed in the regions of cool temperate to tropical latitudes in Asia, Americas, North Africa, and Europe. (Jafari et al., 2018). Oak twigs and leaves are commonly grazed by animals or loped to use as fodder for livestock during feed shortage periods (Singh et al., 1996). Acorns are considered a high energy resource for small ruminants feeding and are often compared to barley (Al Jassim et al., 1998). Boubak et al, 2007 suggested that including oak acorns in concentrate diets of goats can be economically supportive, especially in feed scarcity conditions. Flavonoids, saponins, tannins, essential oils and many other plant secondary compounds from plants or plant extracts have been shown to positively affect rumen

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metabolism, such as decreasing methanogenesis and dietary protein degradation, increasing the production of microbial protein and flow of protein to the duodenum by targeting specific groups of microbial populations in rumen (Wallace, 2004; Patra and Saxena, 2009). Plant secondary metabolites are a diverse group of molecules that play a role in the adaptation of plant to environment (Frutos et al., 2004). Tannins are considered one of the most important secondary metabolites, and are divided into two groups, according to chemical structure: hydrolysable and condensed tannins (Hemingway, 1989). Tannins are polyphenols that directly or indirectly influence feed intake and digestion. They are the primary cause of astringency in feeds, which is the result of its binding to dietary proteins, causing formation of soluble or insoluble complexes (Kumar and Vaithiyanathan, 1990). The degree of the interaction depends highly on the structures of both of polyphenols and feed proteins (Mueller-Harvey, 2006). Oak products contain considerable levels of secondary metabolites (Froutan et al., 2015; Elahi and Rouzbehan, 2008). Barry et al, 1986 concluded that increasing concentration of condensed tannins in sheep diets added advantageous impact on nutritive value by increasing flow of non-ammonia nitrogen and retained nitrogen to duodenum, but this accompanied by detrimental influence in reducing apparent digestibility of organic matter, lignin and energy. However, it is reported that low intake of tannins by small ruminants, might have positive impact mainly on nitrogen metabolism, as low levels of condensed tannins, may form complexes with dietary proteins and lessen their exposure to microbial enzymes (Reed et al., 1990) and protect the soluble proteins from ruminal degradation (Wang et al., 1994) this may lead to increased amount of dietary amino acids flow to the small intestine. It has been concluded by Al Jassim et al, 1998 that acorns could replace 50% of barely in concentrate diets of sheep without any adverse effects. Waghorn, 2008 showed that not all domestic animals are equally affected by the tannins of oak plants, as goats which shown a moderately good performance and were able to utilize oak leaves without any detrimental effects on productivity. In goats, dietary proteins may bind with as much as 50% of the tannin in the diet during ingestion. Goats are mixed feeders which consume considerable amounts of tanniferous feeds (Provenza and Malechek, 1984). The degree of effects of condensed tannins on ruminant performance will depend on their astringency and amount in the diet, nutrient requirements of the animal and the other dietary components (Waghorn, 2008). In ruminant animals, tannins could have advantageous influence if they are included in the diet at moderate levels (Patra and Saxena, 2011). However, Hadjipanayiotou and Antoniou, 1983 reported a significant differences in favor of goats for the microbial protein concentration in the rumen, but they concluded that there are minor differences between goats and ewes for rumen pH, ammonia-nitrogen, total volatile fatty acids concentration and molar proportions of individual volatile fatty acids. When cows and ewes were fed on diets containing less than 40g of tannins /Kg DM, showed a higher retained nitrogen and lower plasma urea concentration, as a result of the ability of tannins to protect dietary proteins from microbial degradation in the rumen (Min et al., 2003; Frutos et al., 2004). It has been suggested by Robbins et al, 1987 that cattle and sheep may be more susceptible to the effects of tannins than browsing ruminants, the reason is that browsing ruminants may have a higher nitrogen retention than grazers, when fed tannineferous feeds. Ruminants may show resistance to feed tannins at different levels; tannin degradation, microbial tolerance and adaptation of intestinal tract(Brooker et al., 1999). Robbins et al, 1991 demonstrated that comparing sheep, mule deer and American black bears in their resistance to tannins, showed that the effect of tannins as digestion inhibitors is related to the animal species. Moreover, Narjisse et al, 1995 evidenced that Moroccan native goats and Moroccan Timahdit sheep exhibited different ranges of resistance to the influence of tannins extract of oak leaves and the higher resistance of goats supported them to maintain their feed intake, nitrogen balance, and rumen fermentation parameters. However, higher level of diet crude protein diminishes most of the detrimental effects of tannins from oak leaves (Singh et al., 1996). The suitable management of natural tannin-containing resources could provide beneficial impact in relation to protein degradation (Bunglavan and Dutta, 2013). The aim of this study was to compare sheep and goats, fed different levels of oak acorns in terms of nutrient digestibility, nitrogen balance, some rumen fermentation parameters and serum biochemical metabolites.

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MATERIALS AND METHODS

This study was conducted at the Animals project of the college of agricultural engineering sciences, University of Duhok. All procedures were approves by the ethics committee of the college of veterinary medicine, University of Duhok.

Chemical analysis

The samples of feed ingredients, experimental diets and feces were analyzed for dry matter, organic matter, crude protein, ether extract and crude fiber content according to the methods shown by AOAC, (2007). The non-protein nitrogen content of samples was determined according to Licitra et al, (1996).

Analysis of oak acorns

The composition of oak acorn is shown in Table 1. The oak acorns were analyzed for proximate analyses according to the methods of AOAC, (2007).The total flavonoids content in acorns was determined according to the method given by Woisky and Salatino, (1998). Quercetin was used as a standard flavonoid, 5 different concentrations of standard quercetin solution in the range of 4-12µg/ml were used to make a calibration curve. To 100 microliter of extract, 1ml of potassium acetate and 1 ml of 5% aluminum chloride were added. The mixture was let to stand for 30 minutes, and then absorbance read at 425nm via spectrophotometer (VersaMax molecular devices, USA). The total phenolics content of oak acorns was determined using Folin-Ciacolteu reagent method as shown by Makkar et al, (1993). A calibration curve was obtained with 5 different concentrations of standard gallic acid solution in the range of 62.5-1000µg/ml. An amount of 0.25ml of Folin-Ciacolteu reagent and 1.25ml of 20% sodium carbonate were added to extract. The mixture was allowed to stand for 45 minutes in darkness, and then the absorbance was read via spectrophotometer at 725nm (VersaMax molecular devices, USA). The content of condensed tannins in acorns was determined according to Makkar et al, (1993) as shown in To 0.2g of dried ground sample, 10ml of 70% aqueous acetone was added and contents kept on ice, and then centrifuged at 3000g for 10 minutes. The supernatant was collected and kept on ice. 0.5ml of supernatant was pipetted to a test tube, 3ml of butanol-HCl reagent and 0.1ml of Ferric reagent, and then put in heating block at 97℃ for 60 minutes. 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)

Collection and analysis of rumen fluid

The samples of rumen fluid were collected using a stomach tube at the 15th day of each experimental period. The pH was recorded immediately at 0, 2, 4 and 6 hours post feeding using a portable pH-meter (Hanna, Hungary), the probe was washed between samplings and the device was calibrated at the beginning of every sampling day by using buffers of pH 4.01 and pH 7.01. The concentration of ammonia-nitrogen in samples was determined using a steam distillation unit followed by titration with 0.01N HCl.

Measurement of digestibility

The nutrient apparent digestibility was measured according to the method shown by McDonald et al, (2010). The digestibility trial consisted of three periods. The first period was an adaptation period. Then, the animals were maintained on the experimental diets for a preliminary period to ensure that the animals were accustomed to the experimental diets and to clear the digestive tract of previous residues. The meals were given to animals at similar times every day. Lastly, the collection period during which the daily feed intake and fecal output were recorded.

Preparation and analysis of blood serum

The blood samples were collected via Jugular venipuncture into plastic vacutainers (Vaccutest, Italy). The samples were stored at 4˚C overnight, and then centrifuged at 4000g for 10minutes. The serum was removed and stored at -18˚C for subsequent analysis. A blood analyzing machine (Cobas, Germany) was used to analyze serum samples.

Preparation of plant material

Oak acorns were collected at Hadena village at Duhok governorate, Kurdistan region, Iraq. To extend the storage period of oak acorns and experimental diets, the oak acorns were stored in shed at 25˚C for 15 days, and then ground to ensure maximum mixing with other feed ingredients in experimental diets.

Preparation of experimental diets

All feedstuffs were sampled and analyzed for DM, OM, CP, EE, CF and NFE. Depending on latter analysis, the raw ingredients were mixed using a mixer machine to formulate the four experimental diets to be T1 (control), T2 (5% oak acorns), T3 (10% oak acorns) and T4 (15%

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oak acorns). The feed ingredients and chemical composition of the experimental diets are presented in Table 2.

Experimental animals and management

In this study, 8 animals were used; 4 rams (live body weight 50± 1 kg) and 4 bucks (live body weight 35± 0.5 kg). The study was conducted over 4 periods each of 15 days. Rams and bucks were allocated to one of the dietary treatments for 15 days; 10 days as adaptation period and 5 days for the collection of samples, and then switched into another dietary treatment (2 animals/ treatment/ period). Every animal was fed on a quantity of dry matter that was equal to 2% of its live body weight. The samples of feces and urine were collected from day 10 to day 15 in metabolism crates, while rumen fluid and blood samples were collected at day 15 of each experimental period.

Statistical analysis

The data of study were analyzed by ANOVA as factorial 2×4 design with the main effects of animal species and level of acorns in the diet using Genstat software (Genstat 17th edition, VSN, UK). The analysis of rumen pH and ammonia-nitrogen concentration data were followed by Fisher's repeated measure analysis of variance using Genstat software (Genstat 17th edition, VSN, UK) in order to determine the effect of time.

RESULTS AND DISCUSSION

Digestibility

The effect of feeding oak acorns on nutrient digestibility in sheep and goats is shown in table 3. There was a significant (P=0.006) differences between sheep and goats in relation to DM digestibility coefficient. Also the rams fed on T3 and T4 had significantly (P≤0.05) lower DM digestibility coefficients as compared to the rams fed on control and bucks fed on all the dietary treatments. The reduction in DM digestibility coefficients of rams fed T3 and T4 as compared to rams fed control may be explained by presence of some anti-nutritional factors in oak acorns. Polyphenols are among the anti-nutritional factors that may exist, which are a group of tannins that are found to be cellulolytic inhibitors which has been reported in many trees and shrubs (Jackson et al., 1996). However, it has been demonstrated that not all tannins or tannins extracted from all plants act similarly on herbivores performance, but their effect is dependent on their chemical structure and molecular weight (Provenza et al., 1990; Hagerman et al., 1992) in addition to the tannins level in the diet, while Jung et al. (1993) reported that feeding condensed tannins at a level more than 5g/kg DM may negatively affect the ruminants performance by reducing the feed intake and digestibility of both of DM and nitrogen. In addition tannins may act to decrease cell wall digestibility through binding bacterial enzymes and/or forming indigestible complexes with cell wall carbohydrates (Barry and Manley, 1986)(Barry et al., 1986). The reduction in DM digestibility coefficient of rams fed T3 and T4 as compared with bucks fed all the experimental treatments may be explained by that goats are more efficient than sheep (Kumar et al., 2014) as in general browsers are more efficient than grazers in consuming tannin-rich plans (Robbins et al., 1991).

The OM digestibility coefficient was significantly (P≤0.05) affected by the species of animal. There was a numerical decrease in OM digestibility coefficient in rams fed T2, T3 and T4 as compared to rams fed control and bucks fed all the dietary treatments, where the rams fed on T3 showed a tendency (P=0.08) for a lower OM digestibility coefficients as compared to the rams and bucks fed on control diet. The higher OM digestibility confidents in rams and bucks fed control diet may be is due to the availability of higher proportion of concentrate mixture in control diet that lead to improve the activity of rumen cellulytic bacteria and causing an increase in nutrient digestibility (Boubak et al., 2007).

There were no effects of dietary treatments on CP and NPN digestibility. The animal species had a trend (P=0.08) to affect CP digestibility coefficient. In this study, the reason of the absence of the effect of experimental treatments on CP and NPN digestibility coefficients may be explained by the absence of effects of treatments on nitrogen retention.

The differences in animal species significantly (P=0.01) affected the CF digestibility coefficient. A numerical decrease in CF digestibility was found in rams and bucks fed dietary levels of acorns. The rams fed on T3 and T4 exhibited a tendency (P=0.07) to have lower CF digestibility coefficients as compared to rams fed control and T2 and bucks fed on all the experimental diets. In the same species, the absence of the effect of experimental treatments on CF digestibility may be related to the fact that the high starch grains in the control diet might led to negative effects on CF digestibility (Boubak et al., 2007).

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The rams and bucks fed on T4 showed a significant (P=0.02) increase in EE digestibility coefficient as compared to that of bucks fed on T2.

Nitrogen balance

The effect of dietary levels of oak acorns on nitrogen balance parameters is presented in table 4. There was a significant effect of animal species on daily digested nitrogen (P<0.001) and retained nitrogen amount (P=0.05), where the bucks fed all dietary treatments exhibited lower amounts of daily digested nitrogen and daily retained nitrogen as compared to the rams fed on control and T4. There was no effect of treatments on nitrogen balance parameters; this may be justified by the probable absence of significant effects of condensed tannins on microbial non-ammonia nitrogen from rumen pool and abomasal flux indicating absence of dietary treatments on total microbial protein production (Min et al., 2002). Same authors indicated that the inhibitory effects of condensed tannins on bacterial proteinase of rumen are species-specific. There are differences in rumen microbial population between sheep and goats (Narjisse et al., 1995). In current study, the apparent digestibility of DM and OM is affected to higher extent in rams than in bucks upon acorns consumption, this may explain the higher nitrogen retention in rams as a result of much formation of complexes between tannins and dietary protein in rumen leading to more flow of limiting amino acids to small intestine (Al Jassim et al., 1998; Wang et al., 1996).

Rumen pH

Table 5. presents the effect of dietary treatments on rumen pH values in rams and bucks. There were no effects of animal species, dietary treatments and interaction between the animal species and level of acorns in the diet, except at the time before the morning feeding, where there was a significant (P=0.03) effect of animal species on rumen pH. The bucks fed on control diet had a significantly lower rumen pH (6.27) as compared to that of rams fed on all the experimental diets (6.86 on average). The lower rumen pH values in bucks as compared to rams may be related to faster feed turnover in the rumen of bucks (Hadjipanayiotou and Antoniou, 1983). In addition lower rumen pH is an indicator of higher production of volatile fatty acids (Woodward and Reed, 1997), in the present study bucks exhibited higher DM digestibility coefficient as compared to that of rams, therefore, the lower rumen pH in bucks may be attributed to higher production of volatile fatty acids which resulted from higher DM degradation in rumen.

Ammonia-nitrogen (NH3-N)

The effect feeding oak acorns on rumen NH3-N concentration in rams and bucks is shown in table 6. The species of animals had a significant (P<0.001) effect on NH3-N concentration before the morning feeding. The lowest concentration of rumen NH3-N before the morning feeding was observed in the rams fed on T3 (4.70mg/dL) which was significantly (P<0.001) lower than that of rams fed T2 and bucks fed the dietary treatments, except for the bucks fed T3 (6.40mg/dL). At 2 hours post feeding, neither the animal species nor the dietary treatments had an effect on rumen NH3-N concentration. At 4 hours post feeding, there was a significant (P=0.006) effect of animal species on rumen NH3-N concentration. The bucks fed T4 exhibited the highest concentration of rumen NH3-N (7.8 mg/dL) which was significantly (P=0.005) that of rams fed T2 and T3. At 6 hours post feeding, both animal species and experimental treatments significantly (P=0.02) affected the rumen NH3-N concentration. The rams fed T3 had a significantly higher rumen NH3-N concentration (5mg/dL) as compared to the rams fed on control diet, while it was significantly than that of bucks fed all the experimental diets. The reason of sharp decline in rumen NH3-N in rams fed T3 and T4 as compared to rams fed control may be due formation of more complexes between acorn secondary metabolites, especially tannins and dietary proteins that results in less degradation of proteins in rumen and this may lead to lower availability of amino acids or reduction in deamination of amino acids (Alexander et al., 2008; Wang et al., 1996)or the reason may be due to reduced protein degradation and protozoal count in the rumen (Wallace et al., 1994). The higher concentration of rumen NH3-N may be explained by the suggestion of Waghorn and Shelton, (1997) that there is a differential effect of condensed tannins on proteolytic bacteria and those digesting soluble carbohydrates that indicates differential effect on the rate of complexes formation between condensed tannins and soluble proteins relative to soluble carbohydrates.

Blood serum parameters

The effect of dietary levels of acorns on serum biochemical characteristics is shown in Table 7. The experimental diets and the

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interaction between animal species and level of acorns in the diet had no effects (P> 0.05) on blood serum metabolites. The serum glucose concentration was significantly (P=0.006) affected by the species of animals. The serum glucose in rams was 78mg/dL on average, while in bucks, in was 69.25mg/dL on average, there was a significant effect of animal species as exhibited in rams fed T3 and T4 represented in higher concentration of serum glucose as compared to bucks fed control and T4 diets. In ruminants, glucose supply to the blood is limited due to extensive ruminal fermentation of carbohydrates; in addition glucose is synthesized by gluconeogenesis from amino acids and propionic acid (Wang et al., 1996). In the present study, the lowest DM digestibility coefficient were found in rams fed T3 and T4, therefore, the higher serum glucose concentrations in rams fed T3 and T4 might be as a result of more glucose been derived from the increased supply of amino acids by gluconeogenesis induced by the formation of more complexes between condensed tannins and dietary proteins in rumen. There were no effects of experimental diets on the concentrations of serum triglycerides and cholesterol in rams and bucks. There are many biologically active phenolic compounds in oak acorns such as tannins, ellagic and gallic acids, and galloyl and hexahydroxydiphenoil derivatives (Rakic et al., 2006) that play a role in the regulation of blood lipid profile (Froutan et al., 2015).

The animal species had a trend on serum protein and urea concentrations. The serum protein concentration in rams was 6.06mg/dL on average and this had a tendency (P=0.04) as compared to that of bucks (5.75mg/dL on average).There was a tendency (P=0.07) in bucks fed T4 as compared to bucks fed T2 and rams fed T3 and control diets. The plasma urea concentration is an indicator of protein degradation in the rumen (Jafari et al., 2018), thus the absence of the effect of dietary treatment on serum urea concentration in the current study may be related to the absence of the effect of treatments on CP digestibility coefficient.

CONCLUSIONS

In the light of the results obtained in this study, it could be concluded that goats have a superior efficiency for digesting diets containing oak acorns as compared to sheep, in addition no toxic effect was evidenced in Karadi sheep and Black goats fed oak acorns at levels up to 15%.

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Table (1). Composition of oak (*Quercus aegilopes*) acorn.

Component	Amount	
DM g/kg	593	
Moisture g/kg	407	
Ash g/kg DM	31.2	
OM g/kg DM	968.8	
EE g/kg DM	87.5	
CP g/kg DM	42	
CF g/kg DM	73.2	
NFE[1] g/kg DM	359.1	
Total Phenolics g/kg DM	Water extract	125.08
	Methanol extract	94.61
Total Flavonoids g/kg DM	Water extract	9.06
	Methanol extract	14.06
Condensed Tannins g/kg DM	3.76	
ME[2] MJ/kg DM		

DM: Dry matter, OM: Organic matter, EE: Ether extract, CP: Crude protein, CF: Crude fiber, NFE: Nitrogen-free extract, ME: Metabolizable energy.

[1]NFE%= 1000- (Moisture + Ash + EE + CP + CF contents).

[2]ME was calculated according to MAFF, (1975), ME= (CP*0.02+EE*0.031+Cf*0.005+NFE*0.014)

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Table (2): Ingredients and chemical composition of experimental diets.

Item	T1	T2	T3	T4
Barley g/kg	500	450	400	400
Wheat bran g/kg	130	120	110	100
Soybean meal g/kg	150	160	170	180
Corn g/kg	100	100	100	50
Wheat straw g/kg	100	100	100	100
Oak acorns g/kg	0	50	100	150
Vits. And minerals. g/kg	10	10	10	10
Iodized Salt g/kg	10	10	10	10
DM g/kg	939.3	937.1	928.9	928.8
OM g/kg DM	934.1	942.1	942.8	931.9
Ash g/kg DM	65.9	57.8	57.1	68.0
CP g/kg DM	148.7	145.2	150.5	152.2
EE g/kg DM	18.3	16.3	22.7	26.8
CF g/kg DM	80.9	79.9	79.5	89.2
NFE[1] g/kg DM	625.4	637.7	619.0	592.3
ME[2]MJ/kg DM	12.7	12.73	12.77	12.61

DM: Dry matter, OM: Organic matter, EE: Ether extract, CP: Crude protein, CF: Crude fiber, NFE: Nitrogen-free extract, ME: Metabolizable energy.

[1] NFE%= 1000- (Moisture + Ash + EE + CP + CF contents)

[2] ME was calculated according to MAFF, (1975); ME= (CP*0.02+EE*0.031+Cf*0.005+NFE*0.014)

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Table (3): Effect of feeding different levels of oak acorns on nutrient digestibility coefficients in sheep and goats.

Coefficient (%)	Species								S ED			P-value		
	Sheep				Goats				Animal	level	Animal* level	Animal	level	Animal* level
	T1	T2	T3	T4	T1	T2	T3	T4						
DM	80.53^{c}	76.62^{abc}	71.80^{a}	72.78^{ab}	82.76^{c}	80.13^{bc}	79.87^{bc}	78.49^{abc}	1.63	2.31	3.26	0.006	0.05	0.61
OM	83.46	79.55	75.32	76.69	84.76	82.04	81.80	80.27	1.68	2.38	3.37	0.05	0.08	0.73
CP	77.45	73.35	69.30	72.64	81.21	74.54	77.49	78.05	2.56	3.62	5.13	0.08	0.37	0.80
CF	55.56	41.02	36.63	34.08	62.82	52.48	51.48	49.57	4.83	6.83	9.66	0.01	0.07	0.92
EE	48.48^{ab}	40.47^{ab}	49.60^{ab}	63.64^{b}	42.25^{ab}	31.10^{a}	49.71^{ab}	57.81^{b}	5.29	7.49	10.59	0.32	0.02	0.93
NPN	78.59	73.93	74.33	76.06	80.03	73.75	78.30	79.36	4.0	5.66	8.01	0.60	0.80	0.98

Different letters within the same row refers to significant difference.

T1: Control, T2: 5% acorns, T3: 10% acorns, T4: 15% acorns, DM: Dry matter, OM: Organic matter, EE: Ether extract, CP: Crude protein, CF: Crude fiber, NFE: Nitrogen-free extract.

Table (4): Effect of feeding oak acorns (different levels) on nitrogen balance parameters in sheep and goats

Parameters	Species								SED			P-value		
	Sheep				Goats				Animal	level	Animal*level	Animal	level	Animal*level
	T1	T2	T3	T4	T1	T2	T3	T4						
Digested nitrogen(g/d)	17.31	13.84	15.38	16.43	12.58	11.33	12.13	12.36	0.82	1.16	1.65	<.001	0.23	0.79
Retained nitrogen (%)	41.26	33.87	43.20	41.53	44.39	34.04	53.19	38.79	6.11	8.64	12.22	0.67	0.43	0.89
Retained nitrogen(g/d)	9.22	7.37	9.60	9.39	6.87	5.19	8.33	6.14	1.11	1.58	2.23	0.05	0.41	0.94
FN/IN (%)	22.55	26.65	30.70	27.36	18.79	25.46	22.51	21.95	2.565	3.62	5.13	0.08	0.37	0.80
UN/IN (%)	36.19	41.93	26.10	31.11	36.82	40.50	24.30	39.26	5.443	7.69	10.88	0.80	0.23	0.90
UN/FN	1.57	2.20	0.99	1.17	2.20	1.64	1.14	1.84	0.30	0.43	0.61	0.47	0.19	0.46

T1: Control, T2: 5% acorns, T3: 10% acorns, T4: 15% acorns, FN: Fecal nitrogen, UN: Urinary nitrogen, IN: Ingested nitrogen.

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Table (5): Effect of different dietary levels of oak acorns on rumen fluid pH in sheep and goats

Time	Species								SED			P-value		
	Sheep				Goats				Animal	level	Animal*level	Animal	level	Animal*level
	T1	T2	T3	T4	T1	T2	T3	T4						
H0	6.83	6.66	6.89	7.07	6.27	6.73	6.75	6.63	0.11	0.16	0.23	0.03	0.29	0.25
H2	6.30	6.09	6.28	6.36	6.13	6.12	6.20	6.21	0.11	0.16	0.23	0.43	0.74	0.93
H4	6.7	6.07	6.42	6.48	6.14	6.10	6.06	6.7	0.12	0.18	0.25	0.14	0.73	0.52
H6	6.21	6.24	6.55	6.53	6.14	6.11	6.51	6.20	0.11	0.15	0.22	0.23	0.10	0.80

T1: Control, T2: 5% acorns, T3: 10% acorns, T4: 15% acorns, H0: Before morning feeding, H2: Two hours post morning feeding, H4: Four hours post morning feeding, H6: Six hours post morning feeding.

Table (6): Effect of different levels of oak acorns on rumen ammonia-nitrogen concentration (mg/dL) in sheep and goats.

Time	Species								S ED			P-value		
	Sheep				Goats				Animal	level	Animal*level	Animal	level	Animal*level
	T1	T2	T3	T4	T1	T2	T3	T4						
H0	7.0^{ab}	7.35^{bc}	4.78^{a}	5.95^{ab}	9.6^{c}	8.2^{bc}	6.4^{ab}	9.4^{c}	0.35	0.49	0.70	<.001	<.001	0.08
H2	5.71	5.48	3.73	4.66	5.48	5.36	6.30	5.95	0.70	0.99	1.41	0.23	0.94	0.47
H4	4.73^{ab}	3.70^{a}	2.36^{a}	3.73^{ab}	7.70^{b}	3.85^{ab}	4.87^{ab}	7.81^{b}	0.73	1.03	1.46	0.006	0.005	0.56
H6	3.50^{bcd}	3.50^{abc}	5.01^{a}	3.15^{ab}	3.26^{d}	7.0^{cd}	6.30^{cd}	4.31^{d}	0.54	0.76	1.08	0.02	0.02	0.15

Different letters within the same row refers to significant difference. T1: Control, T2: 5% acorns, T3: 10% acorns, T4: 15% acorns, H0: Before morning feeding, H2: Two hours post morning feeding, H4: Four hours post morning feeding, H6: Six hours post morning feeding.

Table (7): Effect of different levels of oak acorns on serum metabolites (mg/dL) in sheep and goats.

Parameter	Species								S ED			P-value		
	Sheep				Goats				Animal	level	Animal*level	Animal	level	Animal*level
	T1	T2	T3	T4	T1	T2	T3	T4						
Glucose	75.25	75.25	80.75	80.75	64.25	71.00	75.00	66.75	2.93	4.14	5.86	0.006	0.30	0.62
Protein	6.03	5.97	6.15	6.10	5.70	5.72	5.72	5.87	0.14	0.20	0.28	0.04	0.89	0.96
Albumin	3.07	3.12	3.10	3.15	3.03	2.85	3.08	3.08	0.10	0.14	0.20	0.31	0.83	0.81
Globulin	2.95	2.85	3.05	2.95	2.68	2.85	2.65	2.80	0.13	0.19	0.27	0.14	0.99	0.75
Triglycerides	17.75	17.25	17.50	17.50	18.00	22.00	19.50	19.25	2.97	4.20	5.95	0.46	0.98	0.96
Cholesterol	41.50	44.50	46.75	49.75	44.50	51.00	52.00	60.50	3.96	5.60	7.92	0.12	0.21	0.91
Urea	26.25	29.75	26.25	28.75	28.75	27.00	32.25	34.75	1.57	2.23	3.15	0.07	0.28	0.19
Creatinine	1.00	1.04	0.91	0.97	0.95	0.90	0.91	0.94	0.04	0.05	0.08	0.19	0.62	0.67

T1: Control, T2: 5% acorns, T3: 10% acorns, T4: 15% acorns

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