



Original Research Article

Involvement of Quebracho tannins in diet alters productive and reproductive efficiency of postpartum buffalo cows



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ABSTRACT

This study was conducted to investigate the effects of 10 weeks supplementation of Quebracho tannins (QT; 0 [control], 100 [QT₁₀₀] or 200 g/[cow·d] [QT₂₀₀]) to 30 multiparous postpartum buffalo cows (10 cows per group) on milk yield and composition, blood metabolites and reproductive performance. Supplementation of QT₁₀₀ had no significant effect on milk yield, whereas QT₂₀₀ decreased ($P < 0.05$) this trait. Compared with the control group, both QT levels decreased ($P < 0.05$) fat-corrected milk (FCM) yield, but no significant effects were found on percentages of milk fat and protein. Contrariwise, yields of milk fat, lactose and milk protein were decreased ($P < 0.05$) when QT₂₀₀ was supplemented. The solids nonfat (SNF) percentage and yield were decreased ($P < 0.05$) with QT₁₀₀ supplementation. Moreover, QT tended to numerically reduce total number of ovarian follicles, number of small follicles, peripheral progesterone concentration and conception rate. Supplementation of QT₂₀₀ numerically increased number of large follicles, mean diameter of large follicle, number and diameters of corpora lutea. The inclusion of QT₂₀₀ shortened days open (DO) and decreased number of services per conception. Contrariwise, QT did not show significant effects on serum total protein, albumin, globulin, glucose, cholesterol and triglycerides concentrations. Supplementation of QT₁₀₀ caused an increase ($P < 0.05$) of serum urea compared with that in control and QT₂₀₀ groups. Generally, QT decreased ($P < 0.05$) serum creatinine concentration. Therefore, the supplementation of a commercial QT to early lactating Egyptian buffalo cows displayed negative consequences on their productive and reproductive performances.

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1. Introduction

Buffalo population plays a prominent role in Egyptian rural livestock production system. In the Mediterranean regions, buffalo cows are widely farmed in North Africa and their farming concentrated around the Nile delta for producing milk and beef (Perera, 2011). In spite of buffalo farming spreading out, the

sustainability improvement in milk and meat production is very weak. Indeed, this weakness was basically due to an improvement in farming management tools rather than to genetic selection (Barile, 2005). Reproductive efficiency of buffalo is often compromised by harsh environmental conditions, socio-sexual cues, photoperiod and nutritional status through feed quality and availability (Perera, 2011). Increasing awareness of hazards associated with the use of antibiotic and chemical feed additives (OJEU, 2003), legislators in Europe have moved to prohibit the use of growth-promoting antibiotics in animal feed from the end of 2005 (Marshall and Levy, 2011). The opportunity to exploiting phyto-genic substances e.g. tannins have gained attention in livestock nutrition as natural feed additive alternatives for antibiotics (Bodas et al., 2012) to enhance livestock productivity (Makkar et al., 2009; Sallam et al., 2017). Natural plant compounds, such as condensed tannin (CT) improve nitrogen (N) utilization due to an effective

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rumen escape of dietary protein (Min et al., 2005) and enhance lactation performance and ovulation rate (Min et al., 2003). Additionally, these compounds may prevent reproductive problems in cows that are associated with high level of plasma urea N concentration (Ferguson, 1996). Tannins in high concentrations can be detrimental to reproductive aspects (e.g. ovulation rate, embryo loss and conception rate [CR]) (Min et al., 2001) and to milk production (Gilboa et al., 2000). In the light of shortage of feedstuffs in developing countries, and thus the insufficient nutrient requirements for milk and beef production to meet the over growing human demands, one of the possible avenues to overcome this problem is to look for the efficiency of using tanniferous plants to compensate for such a deficiency. Information on the supplementation of CT extracts in diets of buffalo to assess nutrient utilization, lactational performance and reproductive efficiency is lacking. Thus, our hypothesis was directed to investigate whether Quebracho tannins (QT) supplementation to the diet of milking buffalo cows would have consistent response on productive and reproductive performances.

2. Materials and methods

2.1. Animals and location

This experiment was carried out at a private dairy farm located 75 km south Alexandria. The farm offered 30 multiparous buffalo cows to be utilized in the experiment. All chemical analyses were carried out at the animal nutrition laboratory, Department of Animal and Fish Production, Faculty of Agriculture, Alexandria University. All experimental procedures and sample analyses involving animal care and their use ethics were conducted according to the guidelines approved by the official animal care committee of Alexandria University and in accordance with the Directive 2010/63/EU of the European Parliament and of the Council on the protection of animals used for scientific purposes.

2.2. Diets and experimental design

Thirty postpartum buffalo cows were equally and randomly divided into 3 groups ($n = 10$ cows/group) based on their parities. Buffalo cows were housed in semi-shaded open barns, and had free access to fresh water. Cows were healthy and free from parasites and diseases with a regular protocol of vaccines. They were fed on a concentrate mixture of 6 kg/(cow·d) and alfalfa (*Medicago sativa*) hay *ad lib* according to Ghoneim (1967) without (control, C) or with 100 (QT100) or with 200 g/(cow·d) QT (QT₂₀₀) for a period of 10 consecutive weeks. The QT supplement was mixed well with concentrate mixture daily. The commercial QT was from Argentine (Tanextra A to Industria Argentina). The CT content of the investigated QT was 84.6% according to the method of Yazaki and Hillis (1980). The ingredients and chemical composition of the concentrate mixture and alfalfa provided to buffaloes are shown in Table 1. Animals were fed individually and monitored for their feed consumption, and no feed residues of the concentrate were left before animals at the end of the day.

2.3. Sampling and chemical analysis

Feeds were sampled once biweekly throughout the trial. All samples were dried at 55 °C in a forced-air oven, ground with a Wiley mill grinder to pass through a 1 mm stainless steel screen and then analyzed for DM, OM, EE and CP according to the analytical procedures of the AOAC (2016). However, NDF (Van Soest et al., 1991) and ADF (Robertson and Van Soest, 1981) were determined in sequential analyses using an Ankom fiber analyzer (Fiber Analyzer A200; Ankom Technology, NY, USA). The lactating buffalo

Table 1

Ingredients and chemical composition of the experimental concentrate mixture and alfalfa hay fed to buffalo cows (g/kg, based on DM).¹

Item	Alfalfa hay	Concentrate mixture
Ingredients		
Ground yellow corn		450
Wheat bran		270
Cotton seed meal		250
Limestone		18
Salt		10
Minerals mixture		2
Chemical composition		
OM	914	903
CP	197	178
EE	35	54
NDF	364	436
ADF	287	147
Hemicellulose	77	289
RDP	800	608
RUP	200	392

RDP = rumen degradable protein; RUP = rumen un-degradable protein.

¹ Calculated according to Feedipedia.

cows were milked twice a day at 05:00 and 17:00 using Delaval Bucket Milking System. Milk yield was recorded weekly for each buffalo cow. Milk samples were taken biweekly from morning milking for fat, protein, lactose, solids nonfat (SNF) and ash determinations using the infrared method (EKOMILK-M ultrasonic milk analyzer, EON Trading INC, Bulgaria, 2000). Fat-corrected milk (FCM) yield was calculated as follows:

$$\text{FCM yield} = \text{Milk yield (kg)} \times 0.265 + \text{Milk fat yield (kg)} \times 10.5.$$

Blood samples for sera were collected via tail venipuncture on d 10, 30, 50 and 60 postpartum for blood biochemical parameters assays. Also, blood samples were collected on d 60 (i.e. the first gonadotropin-releasing hormone [GnRH] injection), 67 (i.e. prostaglandin F 2 α [PGF_{2 α}] injection) and 69 (i.e. the second GnRH injection) postpartum for progesterone assay. Blood samples were kept at 4 °C until sera were harvested after being centrifuged at 1,067 \times g for 20 min at 5 °C.

Determined blood biochemical constituents were total protein (Henry, 1974), albumin (Dumas et al., 1970), urea (Young, 1990), glucose (Scheletter and Nussei, 1975), triglycerides (Stein, 1986), cholesterol (Young et al., 1975), and creatinine (Owen et al., 1954). They were determined calorimetrically using commercial kits (Stanbio Diagnostic Company, Germany). Serum globulin concentration was mathematically estimated by subtracting albumin concentration from the total protein concentration. Enzyme-linked immunosorbent assay (ELISA) was used to determine serum progesterone by commercial kits (DiaMetra, Italy). The range of the standards used was 0.0 to 40.0 ng/mL. The intra- and inter assay coefficients of variation (CV) were 4.0% and 9.3%, respectively.

2.4. Ovulation synchronization

Buffalo cows were synchronized for ovulation according to the OvSynch protocol. This involves 2 injections of GnRH analogue (10 μ g Buserelin; i.e. 2.5 mL i.m. Receptal, Intervet International B.V. Boxmeer, Holland) at the 9 d interval and one injection of PGF_{2 α} (500 μ g Cloprostenol; 2 mL i.m., Estrumate, Schering-Plough Animal Health, Germany) given 48 h prior to the second injection of GnRH. Cows were inseminated artificially with frozen semen 16 to 20 h after the second GnRH injection (timed artificial insemination, TAI). The first GnRH injection was given at a random stage of the estrous cycle of lactating cows on d 60 postpartum.

2.5. Ultrasound examination

Ovarian activities and pregnancy diagnoses were examined by using ultrasonography. A real time B-mode scanner (FALCO, USA) equipped with multi frequency (5 and 7.5 MHz) linear array probe was used. Buffalo cows were deprived from feed for 12 h before examination. The probe was pushed gently through the rectum until the anechoic content of the bladder become visible on the monitor then the probe was rotated 90° clockwise and 180° counterclockwise across the reproductive tract until both ovaries and uterine horns were scanned. Diameters and locations of the follicles and corpora lutea on the ovaries were mapped and recorded. Pregnancy diagnosis was carried out on d 35 post insemination. Detection of an anechoic uterus, allantois fluids and/or embryo was considered positive signs of pregnancy. Fig. 1 presents an outline of the time schedule on the experimental buffalo cows.

2.6. Ovarian activities and their implications

Total number of follicles ≥ 2 mm in each ovary in addition to the diameter of each follicle was recorded. Follicles were classified into 3 categories according to their sizes: small (2 to 3 mm), medium (3 to 5 mm) and large (≥ 5 mm). Ovulation was confirmed by the disappearance of large follicle or the presence of luteal structure (CL).

2.7. Reproductive performance

Pregnancy was diagnosed by trans-rectal ultrasonography 35 d after artificial insemination (AI) and confirmed manually by rectal palpation of the uterus from d 40 to 50. Cows returning to estrus were inseminated according to the routine protocol practiced in the farm. Conception rate (CR), overall pregnancy rate (PR), number of services per conception (NSPC) and days open (DO) were recorded. Conception rate (CR) at the first service describes the probability of cows conceiving to the first service (number of pregnant cows divided by inseminated cows). Overall pregnancy rate (PR) describes the number of pregnant cows at the end of the experiment (number of pregnant cows divided by the number of treated cows). Number of services per conception (NSPC) was calculated for individual cows and taken as a mean within each group. Days open is the interval between calving and diagnosed conception.

2.8. Statistical analysis

Statistical analysis for data of blood metabolites and hormone were analyzed as repeated measures analysis of variance using the MIXED procedure of SAS 2002 (Version 9.2) using the following model:

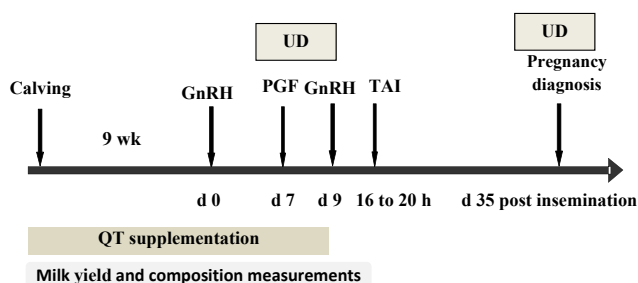


Fig. 1. Time schedule of ovulation synchronization, artificial insemination and pregnancy diagnosis. UD = ultrasound diagnosis; GnRH: gonadotropin-releasing hormone; PGF2 α = prostaglandin F 2 α ; TAI = timed artificial insemination; QT = Quebracho tannins.

$$Y_{ij} = \mu + T_i + D_j + DT_{ij} + E_{ijk},$$

where Y_{ij} = observation, μ = overall mean, T_i = fixed effect of treatment ($i = 1, 2$ or 3), D_j = fixed effect of time ($j = 1, 2$ or 3), DT_{ij} = interaction of treatment \times time and E_{ijk} = residual error assumed to be independent and normally distributed with a mean = 0 and variance = σ^2 . Data of CR, DO and NSPC were analyzed by Chi-square. Multiple comparisons among means were carried out by the Duncan's Multiple Range Test (DMRT, Steel and Torrie, 1980). Level of significance was considered at $P < 0.05$.

3. Results and discussion

Impacts of various level of QT supplementation to buffalo cows at early lactation on milk yield, FCM and milk composition are shown in Table 2. Supplementation of QT₁₀₀ had no significant effect on milk yield but QT₂₀₀ decreased ($P < 0.05$) it compared with the control. Also, supplementation of both QT₁₀₀ and QT₂₀₀ decreased ($P < 0.05$) FCM compared with control. The accumulated effect of QT treatment by time produced a consistent overlapping trend in control and QT₁₀₀. However, QT₂₀₀ treatment resulted in a mild fluctuating decrease in milk yield between wk 2 and 10 of the experiment. Supplementation of QT had no significant effect on milk fat and protein percentages, although their yields decreased ($P < 0.05$) significantly when QT₂₀₀ was provided. The SNF percentage and yield decreased ($P < 0.05$) by supplementing QT₁₀₀ compared with the control group. Moreover, SNF yield decreased ($P < 0.05$) with either dose of QT. The yield of lactose declined ($P < 0.05$) with supplementing QT₂₀₀. Overall time had significant effect on SNF yield, lactose percentage and yield, and milk density. Moreover, there was significant QT \times time interaction on milk yield, milk fat percentage, SNF percentage and yield, lactose percentage and yield, and milk density.

Tannins extract has been suggested as a potentially practical feed additive for protecting dietary protein against rumen microbial degradation. Inclusion of tannins extract in ruminant diet exerts many favorable impacts on ruminant livestock nutrition considering their types, concentrations, chemical structures and physiological status of the consuming species (Hagerman and Butler, 1991). Consuming quantities of tannins showed a magnitude of dosage-dependent effect. Moreover, the effects of CT are not similar as they depend upon their reactivity which is related to their chemical structure (Bueno et al., 2008). Tannins supplementation to ruminant diets depresses feed intake as a result of reduced palatability and decreased rate of digestion (Mueller-Harvey, 2006). Negative effects on milk yield, milk fat and protein values due to the addition of QT to the buffalo diets are consistent with the current results, Wang et al. (1996) observed that inclusion of *Lotus corniculatus* in lactating ewe's diets increased milk yield, milk protein and lactose percentages. These effects might be related to the increased metabolizable protein supply from the protein-binding action of CT (Patra and Saxena, 2011). The current finding of the reduction in milk yield is in agreement with the finding by Maamouri et al. (2011). Several studies have demonstrated that protein protection resulted in an increase of milk yield for cows (Woodward et al., 1999), dairy goats (Rouissi et al., 2006) and sheep (Penning et al., 1988). These findings apparently are inconsistent with the findings of Waghorn et al. (1999) who reported better animal performance with dietary inclusion of CT up to 100 g/kg DM. Moreover, these results in the present study disagree with those of Wang et al. (1996) in dairy sheep and Woodward et al. (1999) in lactating cows who found that milk protein increased with dietary CT supplementation. Inclusion of CT in the diet of dairy ewes had no effect on DM intake, but a tendency of milk yield to decline with

Table 2Effect of various levels of Quebracho tannins (QT) supplementation on milk yield, fat-corrected milk (FCM) yield and milk composition of buffalo cows at early lactation (mean \pm SEM).

Item	Control	QT ₁₀₀	QT ₂₀₀	P-value		
				W	T	W \times T
Milk yield, kg/d	6.9 \pm 0.13 ^a	6.6 \pm 0.14 ^a	5.9 \pm 0.16 ^b	0.1297	0.0001	0.0001
FCM yield, kg/d	6.6 \pm 0.15 ^a	6.0 \pm 0.18 ^b	5.4 \pm 0.19 ^c	0.6237	0.0001	0.3163
Fat percentage, %	6.16 \pm 0.1335	5.93 \pm 0.1547	6.08 \pm 0.1481	0.5095	0.5230	0.0411
Fat yield, kg/d	425.04 \pm 12.848 ^a	391.38 \pm 14.68 ^a	358.72 \pm 14.44 ^b	0.9415	0.0004	0.3123
Protein percentage, %	4.11 \pm 0.1877	4.30 \pm 0.2066	3.94 \pm 0.21889	0.2725	0.4711	0.5502
Protein yield, kg/d	283.59 \pm 14.076 ^a	283.8 \pm 15.517 ^a	232.46 \pm 16.50 ^b	0.3358	0.0233	0.1563
SNF percentage, %	9.50 \pm 0.2019 ^a	8.13 \pm 0.2207 ^b	9.54 \pm 0.2355 ^a	0.0664	0.0001	0.0082
SNF yield, kg/d	657.6 \pm 18.74 ^a	539.58 \pm 20.50 ^b	562.14 \pm 21.97 ^b	0.0068	0.0001	0.0008
Lactose percentage, %	4.97 \pm 0.1061 ^{ab}	5.23 \pm 0.1119 ^a	4.87 \pm 0.1213 ^b	0.0004	0.0519	0.0165
Lactose yield, kg/d	342.22 \pm 9.353 ^a	338.84 \pm 9.951 ^a	285.98 \pm 9.951 ^b	0.0173	0.0002	0.0001
Density, %	31.70 \pm 0.3211 ^{ab}	32.43 \pm 0.3722 ^a	31.36 \pm 0.3745 ^b	0.0001	0.0690	0.0007

SNF = solid non-fat; W = time; T = treatment.

^{a, b, c} Means in the same row with different superscripts significantly differ ($P < 0.05$).

lower milk fat percentage was observed (Molle et al., 2009). These might be related to the type and dose of CT. On the other hand, lower doses of CT varying from 4.5 to 44.5 g/kg DM (Wang et al., 1996; Benchaar et al., 2008) were safely used. These results of high-forage diets provided to buffalo cows are inconsistent with Dschaak et al. (2011) where QT supplementation to dairy cows did not influence milk yield which averaged 34.6 and 36.1 kg/d for the high-forage and low-forage diets, respectively. In addition, formation of QT-protein complex resulted in lower rumen protein degradation and ammonia N (NH₃-N) production, consequently decreased milk urea, but efficiency of N use for milk N was not affected.

Effects of QT supplementation on lactating buffalo cows at an early lactation on follicular dynamics are shown in Table 3. Supplementation of QT₁₀₀ and QT₂₀₀ tended to decrease total number of follicles, number of small follicles and progesterone concentration, but QT₂₀₀ supplementation tended to increase number and diameter of large follicles, and number and diameter of corpus luteum.

Days open, NSPC and CR of lactating buffaloes at early lactation are given in Table 4. The inclusion of QT₂₀₀ shortened DO and decreased NSPC. The inclusion of QT₁₀₀ and QT₂₀₀ reduced CR compared to the control group. Recently, Attia et al. (2016) found negative effects on some reproductive traits due to tannin supplementation in dairy cow diets. There are few studies on the impact of tannins-rich plants or extracts on the reproductive performance of large ruminants, although there are several experiments conducted on the small ruminants. However, short periods of improved nutrient supply before and during mating and reproduction have been known to affect ovulation rate along with increased follicles size and/or number (Bellows et al., 1963), reduced related follicular atresia (Downing and Scaramuzzi,

Table 3Effect of various levels of Quebracho tannins (QT) supplementation on follicular dynamics and progesterone levels of buffalo cows at early lactation (means \pm SEM).

Item	Control	QT ₁₀₀	QT ₂₀₀
TNF	13.44 \pm 1.50	11.20 \pm 1.43	12.22 \pm 1.50
NLF (\geq 5 mm)	5.67 \pm 0.65	5.90 \pm 0.61	6.11 \pm 0.65
MDLF (3 to 5 mm)	10.01 \pm 0.41	9.71 \pm 0.38	10.27 \pm 0.41
NSF (2 to 3 mm)	7.78 \pm 1.36	5.30 \pm 1.29	6.11 \pm 1.36
NCL	0.67 \pm 0.17	0.50 \pm 0.161	1.0 \pm 0.17
DCL, mm	8.91 \pm 2.47	8.11 \pm 2.34	14.98 \pm 2.47
Progesterone, ng/mL	1.46 \pm 0.38	0.52 \pm 0.45	1.30 \pm 0.41

TNF = total number of follicles; NLF = number of large follicles; MDLF = mean diameter of large follicles; NSF = number of small follicles; NCL = number of corpora lutea; DCL = diameter of corpus luteum.

Table 4

Effect of various levels of Quebracho tannins (QT) supplementation on reproductive performance of buffalo cows at early lactation.

Item	Control	QT ₁₀₀	QT ₂₀₀	SEM
Days open, d	121	121	114	4.67
Number of services per conception	2.71	2.71	2.40	0.23
Conception rate, %	66.7	60.0	55.6	—

1991), altered plasma gonadotrophin concentration (Smith, 1988) and increased ovarian sensitivity to gonadotropins (Downing and Scaramuzzi, 1991). These effects probably occur as a result of changes in live weight and body condition, energy and protein intake and absorption from the small intestine (Smith, 1991; Min et al., 1999, 2001), plasma concentration of EAA principally branched chain amino acids (BCAA) (Waghorn, 1986; Waghorn et al., 1990; Downing et al., 1995), and levels of plasma metabolic hormones especially insulin (Downing et al., 1995). A large part of the dietary protein is hydrolyzed in the rumen to ammonia, some of which is re-incorporated into microbial protein. Excess ammonia is absorbed from the rumen and metabolized to urea in the liver, leading to increased plasma ammonia and urea concentrations (Min et al., 2001) which may increase the number of early embryonic losses (El-Zarkouny et al., 2007). In other studies, increased dietary rumen degradable N (RDN) intake has similarly increased plasma urea concentration, leading to increased concentration of ammonia and urea in plasma in the utero-oviductal microenvironment (McEvoy et al., 1997) and uterine secretions (Jordan et al., 1983), decreased uterine pH (Elrod and Buttler, 1993), impaired the viability of sperm (Umezaki and Fordney-Settlage, 1975) and oocyte (O'Callaghan and Boland, 1999), decreased fertilization rate and reduced embryo survival and embryonic development in cows (Blanchard et al., 1990) and ewes (Fahey et al., 2001). Thus, subsequent grazing experiments with sheep showed that CT in *L. corniculatus* increased both ovulation rate and lambing percentage (by 20% to 27%) (Min et al., 1999). These effects of CT were attributed to the reduction in rumen protein degradation to ammonia, increased EAA absorption and reduced early embryonic losses (Min et al., 2001). In New Zealand, an alternative pasture species, *L. corniculatus* has been investigated (Min et al., 2001; Ramirez-Restrepo et al., 2005). The study compared ewes grazing *L. corniculatus* with ewes grazing perennial ryegrass/white clover pasture. It was found that the provision of *L. corniculatus* resulted in a 5% to 33% increase in ovulation rate (maximized if *L. corniculatus* was fed for 2 to 3 estrus cycles before mating), a 6%

Table 5
Effect of various levels of Quebracho tannins (QT) supplementation on blood metabolites of buffalo cows at early lactation (means \pm SEM).

Item	Control	QT ₁₀₀	QT ₂₀₀	P-value		
				W	T	W \times T
Total protein, g/dL	8.21 \pm 0.16	8.24 \pm 0.14	7.92 \pm 0.21	0.0148	0.4438	0.0008
Albumin, g/dL	3.79 \pm 0.12	3.78 \pm 0.11	3.66 \pm 0.16	0.3293	0.7802	0.6082
Globulin, g/dL	4.41 \pm 0.14	4.46 \pm 0.12	4.50 \pm 0.19	0.0404	0.8585	0.0394
Urea, mg/dL	27.94 \pm 1.34 ^b	32.89 \pm 1.15 ^a	25.07 \pm 1.85 ^b	0.0521	0.0004	0.0100
Creatinine, mg/dL	3.07 \pm 0.35 ^a	1.45 \pm 0.34 ^b	2.47 \pm 0.49 ^{ab}	0.8156	0.0039	0.4547
Glucose, mg/dL	53.70 \pm 1.61	53.55 \pm 1.43	55.07 \pm 2.00	0.0280	0.7834	0.5767
Cholesterol, mg/dL	9.36 \pm 0.59	9.09 \pm 0.52	10.84 \pm 0.79	0.0031	0.1303	0.7015
Triglycerides, mg/dL	17.16 \pm 2.25	14.69 \pm 1.91	20.30 \pm 2.92	0.7382	0.2395	0.7000

W = time; T = treatment.

^{a, b} Means in the same row with different superscripts significantly differ ($P < 0.05$).

to 39% increase in lambing percentage, and a 14% to 26% increase in weaning percentage. The effect of *Lotus* on ovulation rate was at least partly dependent on the concentration of active tannins. Other effects might include reductions in the concentrations of rumen and plasma ammonia and plasma urea (Min et al., 2001) or changes in the environment of the oviduct and uterus that are conducive to conception, implantation and fetal development (Ramirez-Restrepo and Barry, 2005). Ultimately, it is of crucial importance to minimize the concentration of tannins to improve the reproductive traits. Therefore, supplementing tannins in high concentrations can be detrimental to reproduction (Min et al., 2001).

Table 5 exhibits data of blood metabolites as a consequence to the supplementation of QT. Quebracho tannins had no significant effect on serum total protein, albumin and globulin concentrations. Time had neither affected total protein nor albumin concentration, but influenced globulin concentration ($P < 0.05$). Supplementation of QT₁₀₀ caused an increase ($P < 0.05$) in the concentration of serum urea compared with the control and QT₂₀₀. There was a significant ($P < 0.05$) time effect on serum urea concentration. Supplementation of QT₁₀₀ significantly decreased ($P < 0.05$) serum creatinine concentration compared with the control. There was no significant effect on serum creatinine concentration by the time during the experimental period. The inclusion of QT had no significant effects on serum glucose, triglycerides and cholesterol concentrations compared with the control. Likewise, there was no significant effect on serum cholesterol concentration due to time period except on d 10 postpartum which revealed significant decrease ($P < 0.05$).

Quebracho tannins effects on blood metabolites in buffalo cows is consistent with the results reported by Blood et al. (1983) who observed that all blood metabolites were within normal ranges in healthy ruminants with no adverse effects on animal performance or health. Supplementation with plant secondary compounds (PSC) can cause some alterations especially in serum insulin (Devant et al., 2007) and plasma glucose decrease (Joy et al., 2001) or increase (Mohammed et al., 2004), hemolysis and anemia (Bernhoft, 2010), and acting as anticoagulants (Dahanukar et al., 2000). An increase in serum urea was reported, however, serum total protein, albumin and creatinine concentrations were not significantly affected when sheep were fed on diets containing CT (Mahgoub et al., 2008). Accordingly, these various responses might result in negative effects on physiological condition as well as physical and chemical consequences. Bioactive components in PSC have been reported to enhance animals immune responses, as an anti-inflammatory agents (Neto et al., 2005), improved humeral and cellular immunity, modulate immune pathways and immune molecules (Rao and Mishra, 1997; More and Pai, 2011). Inclusion of 15% PBP (tannins, 5.5% DM in diet) in early lactating cow's diet had no significant effects on serum glucose, urea and cholesterol

(Rezaenia et al., 2012). Similarly, Bohluli et al. (2009) reported no changes in serum glucose and urea in early lactating dairy cows. Also, Gholizadeh et al. (2010) found that 10% PBP inclusion in the diet of dairy cows did not change serum cholesterol, urea, triglycerides and glucose. In a study in dairy Saanen goats, it was found that the replacement of 30% PBP as a source of alfalfa hay-tannins resulted in unchanged blood metabolites (i.e., cholesterol, triglyceride, urea, total protein, albumin and glucose). Feeding legumes that contained tannins to sheep and goats showed lower plasma urea N (Woodward, 1988). The significant reduction in plasma urea and ruminal NH₃-N reported by Ben Salem et al. (2005) associated with dietary acacia supply. This might be attributed to the reduced proteolysis and decreased microbial proteolytic activity (Jones et al., 1993).

4. Conclusion

Responses to QT as feed additives are dependent upon the type, source and dosage. The culminate outcome of this study indicates little, even none benefits of QT supplementation on productive and reproductive performance of buffalo cows at early lactation. Further studies are required using different proportions of rumen degradable protein and rumen un-degradable protein to confirm these results and to ascertain the mechanisms of QT in ruminant diets.

Conflict of interest

No conflict of interest exists.

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References

- AOAC. Official methods of analysis of AOAC International. 20th ed. Arlington, VA, USA: AOAC; 2016.
- Attia MFA, Nour El-Din ANM, El-Zarkouny SZ, El-Zaiat HM, Zeitoun MM, Sallam SMA. Impact of Quebracho tannins supplementation on productive and reproductive efficiency of dairy cows. OJAS 2016;6:269–88.
- Barile VL. Review article: improving reproductive efficiency in female buffaloes. Livest Prod Sci 2005;92:183–94.
- Bellows RA, Pope AL, Meyer RK, Chapman AB, Casida LE. Physiological mechanisms in nutritionally induced differences in ovarian activity of mature ewes. J Anim Sci 1963;22:93–108.
- Ben Salem H, Makkar HPS, Nefzaoui A, Hassayoun L, Abidi S. Benefit from the association of small amounts of tannin-rich shrub foliage (*Acacia cyanophylla*

- Lindl.) with soya bean meal given as supplements to Barbarian sheep fed on oaten hay. *Anim Feed Sci Technol* 2005;122:173–86.
- Benchaar C, Calsamiglia S, Chaves AV, Fraser GR, Colombatto D, McAllister TA, Beauchemin KA. A review of plant-derived essential oils in ruminant nutrition and production. *Anim Feed Sci Technol* 2008;145:209–28.
- Bernhoft A. A brief review. In: Bernhoft A, editor. *Bioactive compounds in plants-benefits and risks for man and animals*. Oslo, Norway: Norwegian Acad. of Sci. and Letters; 2010. p. 11–7.
- Blanchard T, Ferguson J, Love L, Takeda T, Henderson B, Hasler J, Chalupa W. Effect of dietary crude-protein type on fertilization and embryo quality in dairy cattle. *Am J Vet Res* 1990;51:905–8.
- Blood PC, Radostits OM, Henderson JA, Arundel JH, Gay CC. *Veterinary medicine*. 6th ed. Eastbourne BN 21 3UN: ELBS and Bailliere, Tirirad; 1983. p. 260–70.
- Bodas R, Prieto N, García-González R, Andrés S, Giráldez FJ, López S. Manipulation of rumen fermentation and methane production with plant secondary metabolites. *Anim Feed Sci Technol* 2012;176:78–93.
- Bohluli A, Naserian A, Valizadeh R, Eftekhari F. The effect of pistachio by-product on nutrient apparent digestibility, rumination activity and performance of Holstein dairy cows in early lactation. *JWSS* 2009;13(47):167–79.
- Bueno LCS, Vitti DMS, Louvandini SH, Abdalla AL. A new approach for in vitro bioassay to measure tannin biological effects based on a gas production technique. *Anim Feed Sci Technol* 2008;141:153–70.
- Dahanukar SA, Kulkarni RA, Rege NN. *Pharmacology of medicinal plants and natural products*. *Ind J Pharmacol* 2000;32:S81–118.
- Devant M, Anglada A, Bach A. Effects of plant extracts supplementation on rumenfermentation and metabolism in young Holstein bulls consuming high levels of concentrate. *Anim Feed Sci Technol* 2007;137:46–57.
- Doumas BT, Watson WA, Biggs HG. Albumin standards and the measurement of serum albumin with bromocresol green. *Clin Chim Acta* 1970;31:87–96.
- Downing JA, Scaramuzzi RJ. Nutrient effects on ovulation rate, ovarian function and the secretion of gonadotrophic and metabolic hormones. *J Reprod Fertil* 1991;43:209–27.
- Downing JA, Joss J, Scaramuzzi RJ. A mixture of the branched chain amino acids leucine, isoleucine and valine increases ovulation rate in ewes when infused during the late luteal phase of the estrus cycle: an effect that may be mediated by insulin. *J Endocrinol* 1995;145:315–23.
- Dschaak CM, Williams CM, Holt MS, Eun JS, Young AJ, Min BR. Effects of supplementing condensed tannin extract on intake, digestion, ruminal fermentation, and milk production of lactating dairy cows. *J Dairy Sci* 2011;94:2508–19.
- Elrod CC, Buttler WR. Reduction of fertility and alteration of uterine pH in heifers fed excess ruminally degradable protein. *J Anim Sci* 1993;71:694–701.
- El-Zarkouny SZ, Ghobashy H, Nour El-Din ANM, Abdel-Gauad EI, Hassan GA. Lambing and embryonic mortality rates in Awassi and Barki ewes and their crosses throughout three different mating seasons in the west coastal region of Egypt. *Alexandria J Agric Res* 2007;52(3):15–24.
- Fahey J, Boland MP, O'Callaghan DO. The effects of dietary urea on embryo development in superovulated donor ewes and on embryo survival following transfer in recipient ewes. *Proc. Br. Soc. Anim Sci* 2001;72(2):395–400.
- Ferguson JD. Diet, production and reproduction in dairy cows. *Anim. Feed Sci Technol* 1996;59:173–84.
- Gholizadeh H, Naserian AA, Valizadeh R, Tahmasebi A. Effect of feeding pistachio-byproduct on performance and blood metabolites in Holstein dairy cows. *Int J Agric Biol* 2010;12:867–70.
- Ghoneim A. *Animal nutrition (feeding requirements and Economic ration)*. Cairo, Egypt: Anglo Press Library (In Arabic); 1967.
- Gilboa, Perevolotsky A, Landau S, Nitsan Z, Silanikove N. Increasing productivity in Goats grazing Mediterranean woodland and scrubland by supplementation of Polyethylene glycol. *Small Rumin Res* 2000;38:183–90.
- Hagerman AE, Butler LG. Tannins and lignins. In: Rosenthal GA, Berenbaum MR, editors. *Herbivores: their interactions with secondary plant metabolites, Vol I: the chemical participants*. NY (USA): Academic Press; 1991. p. 355–88.
- Henry JB, Todd, Sanford, Davidsohn: "Clinical diagnosis and measurement by laboratory methods". 16th ed. Philadelphia P.A.P: W.B. Saunders and Co; 1974. p. 260–4.
- Jones GA, Jakober KD, Bae HD, McAllister TA, Cheng KJ. Some interactions between condensed tannins of forage legumes, bovine serum albumin, and five strains of proteolytic rumen bacteria. *Proceedings of the World Conference on Animal Production, Edmonton, Canada* 1993;36:68–9.
- Jordan ER, Chapman TE, Holtan DW, Swanson LV. Relation of dietary crude protein to composition of uterine secretions and blood in high-producing postpartum dairy cows. *J Dairy Sci* 1983;66:1854–62.
- Joy PP, Thomas J, Mathew S, Skaria BP. *Medicinal plants*. In: Bose TK, Kabir J, Das P, Joy PP, editors. *Tropical horticulture*. Calcutta, India: Naya Prokash; 2001. p. 449–632.
- Maamouri O, Atti N, Kraiem K, Mahouachi M. Effects of concentrate and Acacia-cyanophylla foliage supplementation on nitrogen balance and milk production of grazing ewes. *Livest Sci* 2011;139:264–70.
- Mahgoub O, Kadim IT, Tageldin MH, Al-Marzooqi WS, Khalaf SQ, Ambu Ali A. Clinical profile of sheep fed non-conventional feeds containing phenols and condensed tannins. *Small Rumin Res* 2008;78:115–22.
- Makkar HPS, Norsambuu T, Lkhagvatseren S, Becker K. Plant secondary metabolites in some medicinal plants of Mongolia used for enhancing animal health and production. *Tropicultura* 2009;27(3):159–67.
- Marshall BM, Levy SB. Food animals and antimicrobials: impact on human health. *Clin Microbiol Rev* 2011;24(4):718–33.
- McEvoy TG, Robinson JJ, Aitken RP, Findlay PA, Robertson IS. Dietary excesses of urea influence the viability and metabolism of preimplantation sheep embryos and may affect fetal growth among survivors. *Anim Reprod Sci* 1997;47:71–90.
- Min BR, Attwood GT, McNabb WC, Barry TN. Effect of condensed tannins on proteolytic bacterial populations in the rumen and on nitrogen flow to the abomasum of sheep. *J Anim Sci* 2001;79:163.
- Min BR, Attwood GT, McNabb WC, Molan AL, Barry TN. The effect of condensed tannins from *Lotus corniculatus* on the proteolytic activities and growth of rumen bacteria. *Anim Feed Sci Technol* 2005;121:45–58.
- Min BR, Barry TN, Attwood GT, McNabb WC. The effect of condensed tannins on the nutrition and health of ruminants fed fresh temperate forages: a Review. *Anim Feed Sci Technol* 2003;106:3–19.
- Min BR, McNabb WC, Barry TN, Kemp PD, Waghorn GC, McDonald MF. The effect of condensed tannins in *Lotus corniculatus* upon reproductive efficiency and wool production in sheep during late summer and autumn. *J Agric Sci Cam* 1999;132:323–34.
- Mohammed N, Ajisaka N, Lila ZA, Hara K, Mikuni K, Kanda S, Itabashi H. Effect of Japanese horseradish oil on methane production and ruminal fermentation in vitro and in steers. *J Anim Sci* 2004;82:1839–46.
- Molle G, Decandia M, Giovanetti V, Cabiddu A, Fois N, Sitzia M. Responses to condensed tannins of flowering sulla (*Hedysarum coronarium* L.) grazed by dairy sheep. Part 1: effects on feeding behavior, intake, diet digestibility and performance. *Livest Sci* 2009;123:138–46.
- More P, Pai K. Immunomodulatory effects of *Tinospora cordifolia* (Guduchi) on macrophage activation. *Biol Med* 2011;3:134–40.
- Mueller-Harvey I. Unraveling the conundrum of tannins in animal nutrition and health. *J Sci Food Agric* 2006;86:2010–37.
- Neto AG, Costa JM, Belati CC, Vinholis AH, Possebom LS, Da Silva Filho AA, Cunha WR, Carvalho JC, Bastos JK, Silva ML. Analgesic and anti-inflammatory activity of a crude root extract of *Pfaffia glomerata* (Spreng) Pedersen. *J Ethnopharmacol* 2005;96:87–91.
- O'Callaghan DO, Boland MP. Nutritional effects on ovulation, embryo development and the establishment of pregnancy in ruminants. *Anim Sci* 1999;68:299–314.
- OJEU. Regulation (EC) No. 1831/2003 of European Parliament and the Council of 22 September 2003 on additives for use in animal nutrition. Brussels, Belgium: Off J Eur Union; 2003. Page L268/36 in OJEU of 10/18/2003.
- Owen JA, Iggo B, Scandrett JF, Steward CP. The determination of creatinine in plasma or serum and in urine: a critical examination. *Biochem J* 1954;58:426–37.
- Patra AK, Saxena J. Exploitation of dietary tannins to improve rumen metabolism and ruminant nutrition. *J Sci Food Agric* 2011;91:24–37.
- Penning PD, Orr RJ, Treacher TT. Responses of lactating ewes offered fresh herbage indoors and when grazing, to supplements containing differing protein concentrations. *Anim Prod* 1988;46(3):403–15.
- Perera BMAO. Reproductive cycles of buffalo. *Anim Reprod Sci* 2011;124:194–9.
- Ramirez-Restrepo CA, Barry TN. Alternative temperate forages containing secondary compounds for improving sustainable productivity in grazing ruminants. *Anim Feed Sci Technol* 2005;120:179–201.
- Ramirez-Restrepo CA, Barry TN, Lopez-Villalobos N, Kemp N, Harvey TG. Use of *Lotus corniculatus* containing condensed tannins to increase reproductive efficiency in ewes under commercial dryland farming conditions. *Anim Feed Sci Technol* 2005;121:23–43.
- Rao KS, Mishra SH. Anti-inflammatory and hepatoprotective activities of *Sida rhombifolia* Linn. *Indian J Pharmacol* 1997;29:110–6.
- Rezaeiana A, Naserian AA, Valizadeh R, Tahmasbi A. Effect of using different levels of pistachio by-products silage on composition and blood parameters of Holstein dairy cows. *Afr J Biotechnol* 2012;11:6192–6.
- Robertson JB, Van Soest PJ. The detergent System of analysis. In: James WPT, Theander O, editors. *The analysis of dietary fiber in food*, chapter 9. New York: Marcel Dekker; 1981. p. 123–58.
- Rouissi H, Atti N, Mahouachi M, Rekkik B. Effet de la complementation azotée sur les performances zootechniques de la chèvre locale. *Tropicultura* 2006;24:111–4.
- Sallam SMA, El-Gendy MN, Anwar MM, Fahmy WG, El-Zarkouny SZ, Hashem NM, Nour El-Din ANM, Attia MFA, El-wakeel EA, Zeitoun MM. Productive and reproductive traits of sheep fed Acacia saligna Leaves-based diets. *IJEAB* 2017;2(6):2829–40.
- SAS. SAS users guide Statistical Analyses. Cary, USA: Systems Institute; 2002. p. 549–640.
- Scheletter G, Nusse E. *Arbeitsmed, Sozialmed, Praventivmed* 1975;10:25.
- Smith JF. Influence of nutrition on ovulation rate in the Ewe. *Aust J Biol Sci* 1988;41:27–36.
- Smith JF. A review of recent developments on the effect of nutrition on ovulation rate (the flushing effects) with particular reference to research at Ruakura. *Proc NZ Soc Anim Prod* 1991;51:15–23.
- Steel RGD, Torrie JH. *Principles and procedures of Statistics*. NY, USA: McGraw-Hill Book Company Inc; 1980.
- Stein EA. In: Tietz NW, editor. *Textbook of clinical chemistry*. Philadelphia: W. B. Saunders; 1986. p. 879–86. 1818–1829.
- Umezaki C, Fordney-Settlage DS. In vitro studies on cervical contraception: use of urea as spermicidal agent. *Contracept* 1975;12:465–76.
- Van Soest PJ, Robertson JB, Lewis BA. Methods for dietary fiber, neutral detergent fiber and non-starch polysaccharides in relation to animal nutrition. *J Dairy Sci* 1991;74:3583–97.

- Waghorn GC. The effect of different protein/energy intakes on nutritional and physiological parameters in young sheep. *Proc NZ Soc Anim Prod* 1986;46:31–5.
- Waghorn GC, Jones WT, Shelton ID, McNabb WC. Condensed tannins and the nutritive value of herbage. *Proc NZ Grassland Soc* 1990;51:171–6.
- Waghorn GC, Reed JD, Ndlovu LR. Condensed tannins and herbivore nutrition. In: Baile LD, McCaughey P, editors. *Proceedings of the XVIII international grasslands congress*. Buchanan-smith JG. Calgary, Alberta: Association Management Centre; 1999. p. 153–66.
- Wang Y, Douglas GB, Waghorn GC, Barry TN, Foote AG. The effect of condensed tannins in *Lotus corniculatus* upon lactation performance in ewes. *J Agric Sci Camb* 1996;126:353–62.
- Woodward A. Chemical composition of browse in relation to relative consumption of species and nitrogen metabolism of livestock in southern Ethiopia. Ph.D. Dissertation. Ithaca, NY: Cornell University; 1988.
- Woodward SL, Auldred MJ, Laboyrie PJ, Jansen EBL. Effect of *Lotus corniculatus* and condensed tannins on milk yield and milk composition of dairy cows. *Proc NZ Soc Anim Prod* 1999;59:152–5.
- Yazaki Y, Hillis WE. Molecular size distribution of radiate pine loark extracts and its effect on properties. *Holzforschung* 1980;34:125–30.
- Young DS. Effects of drugs on clinical laboratory tests. 3rd ed., vol. 3; 1990. p. 168–82.
- Young DS, Pestaner LC, Gibberman V. Effects of drugs on clinical laboratory tests [Special Issue]. *Clin Chem* 1975;21(5):1D–432D.