Performance and Milk Anti-Oxidant Property of Dairy Goats Fed Pomegranate Seed Pulp and Soybean Oil

Jongkolnee Yaowapaksophon
Department of Animal Production Technology and Fisheries, Faculty of Agricultural Technology, King Mongkut's Institute of Technology Ladkrabang, Bangkok, Thailand
Email: jongkolnee.ya@kmitl.ac.th

Abstract—**The objective of this research was to investigate the effects of supplementing two feedstuffs, Pomegranate Seed Pulp (PSP) as a source of plant secondary compounds or Soybean Oil (SO) as an energy source in order to enhance production performance and milk quality of Saanen dairy goats. Eight lactating cross-bred goats were assigned to receive diets in a replicated 4 × 4 Latin square design with 4 periods of 14 d adaptation and 7 d of data and sample collection. The four dietary treatments were unsupplementation (CON), supplementation with dried Pomegranate Seed Pulp (PSP) at 120 g/kgDM, supplementation with soybean oil (SO) at 50 g/kgDM, and supplementation with SO at 50g/kgDM and PSP at 120 g/kgDM (PSPSO), of concentrate diets. The dietary treatments with similar iso-nitrogenous (166 g/kgDM) and forage to concentrate ratio (36:64) were used. BW change, DM intake, protein intake, total tract apparent nutrient digestibility, milk yield, lactose, solid-not fat, total solid content and yield and solid-corrected yield of goats were not affected (P>0.05) by dietary treatments. Intake of PSPSO diet by PSPSO goats appeared to improve components and yields of milk fat, protein, 4%fat-corrected milk yield, milk efficiency (FCM/DM intake), responsibility of marginal FCM yield and milk flanoids (P=0.05). Somatic cell count of goat milk (520,000 cells/ml) was decreased (P<0.01) by the use of PSPSO diet. In conclusion use of combination between dried Pomegranate Seed Pulp and Soybean Oil (PSPSO) of goat diet could increase milk protein content and yield, milk fat content and yield, fat corrected yield, fat quality and milk oxidative stability property.**

Index Terms—**pomegranate seed pulp, soybean oil, quality and quantity of goat milk, milk antioxidant capacity**

I. Introduction

Pomegranate (*Punica granatum* L.) has exert beneficial advantages, is an attractive source of bioactive compounds, natural food preservatives. Pomegranate medicinal bioactive ingredients include several groups of phytochemical compounds [1], [2], in particular, phenolic compounds which have high correlation with disease-fighting compounds and natural antioxidant capacity, help to maintain human health to preventing diseases [3]. Pomegranate ingredients are used as nutraceuticals [3], herbal medicines, and pharmaceutical formulations. Polyphenolic ingredients and hydrolysable tannins of pomegranate including ellagic tannins, ellagic acid, punicalin, punicalagin and gallic acid which have been shown properties of pathogen inhibition both *in situ* and *in vitro*. Pomegranate multi-functionality ingredients inhibit growth of prostate and breast cancer cells [4], [5], exhibit high activities of antibacterial, anti-inflammatory, anti-allergic [6], anti-mutagenic, anti-hypertension [7], immunomodulatory [8], neuroprotective property [9] and skin protection to UVB exposure [5]. In Thailand Pomegranate Seed Pulp (PSP) is by-products from small holding agriculture, household sector and pomegranate juice vender. Pomegranate Seeds Pulp (PSPs) are by-products of extracting the juice from pomegranates. These by-products, PSPs, may have the potential to be a good source of nutrients for livestock feeds at cheap prices and to reduce the cost of animal production [10]-[18]. As alternative feed resource, the pomegranate by-products antioxidant polyphenol-rich could be used as new raw material of dietary nutrient in support of milk production. Thus, PSP's untapped potential was encouraged the formation of this trial on value-addition capacity of feedstock PSP waste.

Supplemental lipids in goat feed formulations are used to increase the rations energy density and for developing nutritional or economic strategy. Dietary lipid manipulation could enhance the nutritional and energy quality of goat milk [19]-[22], to increases proportions of Monounsaturated Fatty Acids (MUFAs) and Polyunsaturated Fatty Acids (PUFAs) in milk fat, thereby improving health of human consumers [19]. Dietary manipulation by feeding plant oil rich in either linoleic acid (C18:2, LA) or alpha-linolenic acid (C18:3, ALA), soybean oil (SO), has been shown to increase the functional bioactive fatty acids in milk and healthy fat [20]-[22]. However, this may increase the occurrence of spontaneous oxidized deterioration flavor in milk. The presence of natural antioxidants in pomegranate seed pulp, such as polyphenols, may inhibit milk fat oxidation. Scientific assessment effects of Pomegranate Seed Pulp (PSP) and Soybean Oil (SO) of dairy goat diets on milk fatty acid profile and animal health beneficial phytochemicals and milk antioxidant capacity are lacking.

Manuscript received September 24, 2017; revised January 29, 2018.

doi: 10.18178/joaat.5.2.109-116

Very little research has addressed these combination effects of these powerful ingredients (PSPSO) on productive performance of goat.

The objective of this research was to investigate the effects of supplementing two feedstuffs, dried pomegranate seed pulp as a replacement of fine rice bran and partial replacement of broken rice in the diets, or soybean oil as an energy source in order to improve nutrient digestibility, quality and production of goats.

II. Material and Methods

A. Research Location, Animals, Diets, Experimental Design, Digestibilities, Facilities and Managements

The experiment was conducted at the private research farm in geographic location of the Faculty of Agricultural Technology, KMITL, Bangkok, Thailand between the years of 2010 and 2012. Eight healthy multiparous lactating crossbred Saanen dairy goats were used in this study. The animals, averaging 41±1.5 kg body weight (BW), 48±2.1 days in milk (DIM), and producing 1.98±0.35 kg of milk/d at the beginning of the trial, were balanced and were divided into 2 groups of 8. Within each group, goats were exposed to each 4 treatments using replicated 4X4 Williams Latin square design. Or each experimental period, within square, goats were randomly assigned to a sequence of four diets during each of the four 21-d periods (14 d of diet adaptations and 7 d of sample collections).

The four dietary treatments were designated as follows: 1) control diet/unsupplementation (CON), 2) control diet and supplemented with dried pomegranate seeds pulp (PSP) at 120 g/kgDM, 3) control diet and supplemented with soybean oil (SO) at 50 g/kgDM, and 4) control diet and supplemented with SO at 50 g/kgDM and PSP at 120 g/kgDM (PSPSO) of concentrate diet. In this experiment pomegranate fruits and soybean are commonly consumed and commercially grown in Thailand, and use the pomegranate seeds pulp by-product from small scale agribusiness sector and local private holder and soybean oil from soybean oil agro-industrial manufacturer.

The basal diet was Finger hay (*Digitaria eriantha* cv. Taiwan) and the concentrate diets were prepared every week. The diets were compounded to be iso-nitrogenous (166 g/kgDM). The CON and PSP diets were isoenergetic (2.4 Mcal/kgDM), and the SO and PSPSO were isoenergetic (2.6 Mcal/kgDM). The experimental diets were based on 36% roughage and 64% concentrates (including either 120 g/kgDM PSP or 50 g/kgDM SO) and formulated to meet the nutrient requirement of dairy goats. Levels of broken rice and soybean meal ingredient were adjusted slightly to ensure that diets were iso-proteic (166 g/kgDM). The nutritional management and feed formulations were formulated according to the recommendations of the Bureau of Animal Nutrition Development (BAND, 2010), Livestock Development Department of Thailand (DLD) [23]. The proportion of the ingredients of the diets are presented in Table I. Concentrate mixtures contained ingredients as ground maize, broken rice, rice bran, soybean meal, dried pomegranate seed pulp, soybean oil, bone meal, sodium chloride, and mineral–vitamin mix (each kg of the vitamin–mineral premix contained (DM basis): vitaminA (50000IU), vitamin3 (10000IU), vitaminE (0.1g), calcium (196g), phosphorus (96g), sodium (71g), magnesium (19g), iron (3g), copper (0.3 g), manganese (2g), zinc (3g), cobalt (0.1g), iodine (0.1g), selenium (0.001g).

Body weights were measured daily during the sampling period prior to feeding. Subsequent weights were taken every 21 days. Hay was offered *ad libitum*. The concentrates were accessible as two equal meals at 06:30 and 14:30 h; the experimental diets were given to feed refusals between 5 and 10% of the total concentrate offered on as fed-basis.

TABLE I. Ingredients of Concentrate Diets[1] Fed to Lactating Crossbred Dairy Goats (G/Kg Dm)

Foodstuffs	CON	PSP	SO	PSPSO
Maize grain, ground	248	245	236	235
Broken rice	208	185	160	140
Fine rice bran	100	0	100	0
Soybean meal (446 g/kg CP solvent extract)	400	406	410	411
Soybean oil	0	0	50	50
Pomegranate seeds pulp[5]	0	120	0	120
Bone meal	18	18	18	18
Mineral vitamin mix	18	18	18	18
Sodium chloride	8	8	8	8

[1] One sample obtained from eight pool samples prepared by compositing seven daily samples.

[2] The CP, EE, NDF, ADF, total phenols, total tannins of PSP were 113, 108, 440, 320, 42 and 33 (g/kg of DM) respectively.

The conventional method of *in vivo* digestibility was used in this study. Digestibility level of the whole body animal was the total tract apparent nutrient digestibility (TTAD) [24]. Nutrient TTAD were measured using acid-insoluble ash as an internal maker. Thus, during the last 7 days of each period, feed consumption measurements, feed refusals, and total collection of feces per day were sampled daily in the collection period and pooled within period. Feces and feed refusals were collected daily, before the morning feeding, of each collection period and pooled within goat and period. Only 10% of total feces were kept and all diet samples were frozen at −20 ℃ for subsequent drying at 55 ℃. All samples were ground through a 1 mm screen in a Wiley mill for further analyses.

Before p.m. milking, the concentrate diet was placed for control hay intake. The animals were housed in individual stalls to support individual feed consumption. The animals were allowed free access to fresh water. The goats were milked at 07:30 and 15:30 h and milk yield was recorded at each milking. Two consecutive milk samples of both the a.m. and p.m. milkings were pooled daily or were composted on a proportional weight basis per milking time. The samples were split into 2 aliquots.

The first aliquot was not added for preservation of milk samples. For the second aliquot of milk, sodium azide tablet was added for preservation of milk samples, for one tablet contains 8 mg sodium azide [25]. The milk samples were stored in ice boxes or under refrigeration and transported to our laboratory and the second aliquot kept at 4℃ until analysis for milk components and somatic cell count (SCC). For the first aliquot of milk was stored at −20°C until it was analyzed for fatty acid profile by GLC analysis (result of fatty acid profile was not presented in this paper).

TABLE II. NUTRIENT PROFILE OF EXPERIMENTAL DIETS[1, 2] FED TO LACTATING CROSSBRED SAANEN DAIRY GOATS

Nutrient profile	Experimental treatments (g/kg DM)			
	CON	PSP	SO	PSPSO
CP	166.39	166.93	166.21	165.58
EE	24.35	23.47	51.85	51.02
Ash	56.05	52.33	53.93	50.19
NDF	3263.03	3465.08	3231.55	3432.60
ADF	1848.95	2030.16	1840.97	2020.50
NFC	3968.08	3804.03	3703.58	3552.99
ME (Mcal/ kgDM)	2.46	2.43	2.62	2.59

1 Base on the dietary treatments consisted of hay and concentrates with a F/C=36/64 or intake of hay 360 g/kg DM basis and concentrate 640 g/kg DM basis.

2 BAND, DLD (2010), tabular values of ingredients.

B. Gross Chemical Analysis of Feeds and Feces, and Calculation of Energy Values, Measurements of Total Milk Components

Diet samples were analyzed for moisture (Method No: 934.01), ash (Method No: 942.05), crude fat (Method No:920.39) and total protein contents (CP, the Kjeldahl N × 6.25, Method No: 990.03) according to AOAC system [26]. The cell wall analysis, Neutral Detergent Fiber (NDF) and Acid Detergent Fiber (ADF) content of samples were analyzed according to Van Soest system [27]. The metabolizable energy (ME) contents of diets were estimated from their chemical composition following the equation of [28]; ME (Mcal/kgDM) = 0.012 CP + 0.031 EE + 0.005 CF + 0.014 NFE. The non-fibrous carbohydrates (NFC) were calculated according to the equation; NFC (g/ kgDM) = 1000 (g/kg) − (g/kg NDF + g/kg CP + g/kg EE + g/kg Ash). Chemical composition details and calculation of energy values of the experimental diets were provided in Table II. Intake of NFC, NDF and intake of the NFC:NDF ratio were calculated, as illustrated in Table III. Measurements of milk commercial traits by milk content analyzer (LactoStar: 3510-072003, Funke Gerber, Berlin, Germany) after appropriate calibration of the instrument were made. The milk samples were used to measure somatic cell count ($\times 10^3$ cells/ml) using a Foss Electric Fossomatic cell counter.

C. Preparation of Crude Methanol Extracts from Dried Diet Sample or Milk, Determination of Phenolics and Flavonoids

Preparation of crude methanol extracts of phenolics from dried diet sample or milk are influenced by their chemical nature, liquid or solid state, extraction method applied, and presence of interfering substances. According to that, it is application procedure that is an appropriateness for phenolics extraction in different state of samples and food matrixes. All polyphenols were determined by using the method described in [29]-[31] by Folin-Ciocalteau. Total phenols results were expressed as gallic acid equivalents (GAE, mg/l milk and mg/kg feed) Non-tannin phenols obtained after precipitating with polyvinyl polypyrrolidone. Total tannins were estimated from the difference between total phenols and non-tannin phenolics. Flavonoids were determined by using the method described in [32]. Concentrations of flavonoids were expressed as quercetin equivalents (QE, mg/l milk).

D. Statistical Analysis

All data obtained from the experiment were statistically analyzed according to a repeated 4×4 Latin square design using model of SAS package. Data of feed intake, milk production & composition, and efficien- cy, milk fatty acid, milk phenolic were analyzed using MIXED model, but with sample time as a repeated measure. Goat, period and goat by period were considered as random effects. Effects of dietary treatments were considered as fixed effects. Least squares means (LS MEANS) were computed and detected the difference between dietary treatments. LS comparisons means of the different treatments were done using Fisher's protected LSD (least significant difference) adjustment for the probability. Effects of dietary treatments are declared significant $P \leq 0.05$, and trends are declared at $0.05 < P \leq 0.10$.

III. RESULTS AND DISCUSSION

A. Feed Intake and Nutrient Digestibility

Dietary CP content was similar among treatments. NDF was 6.21% higher in PSP and PSPSO diets compared to the CON diet and SO diet. NFC content was the lowest in PSPSO diet (3,553g/kgDM) and NFC content was the highest in CON diet (3,968g/kgDM). The ether extract level of the diets increased from 23g/kgDM (CON diet and PSP diet) to 67g/kgDM (diets containing soybean oil or pomegranate seed pulp). Consequently, the diets containing oil (SO and PSPSO diets) had a higher (2.61Mcal/kgDM) concentration of metabolizable energy (ME) in relation to the CON diet and PSP diet (2.44 Mcal/kgDM).

The mean of BW, DM intake, and nutrients intake of goats are presented in Table III. The supplementation of pomegranate seed pulp or soybean oil in the experimental diets did not affect ($P>0.10$) the variation of the goat's body weight, DM intake, DM intake/$BW^{0.75}$ and protein intake. The BW ranged from 46.85 to 47.88kg. Supplementation of PSP or SO in goat feeds had no effect on BW change which indicated that the energy for milk production had been met by the energy in the diets [23]. Experimental diets were isocaloric across treaments, averaging 2.5Mcal/kgDM and were formu- lated to meet requirement. In contrast, [12] demonstrated that dietary supplementation with fresh pomegranate peels tended to increase BWgain in bull calves. Average daily DM intake ranged from 1,594 to 1,625g/day and protein intake ranged from 299.96 to 302.41g/day. In agreement with our study, [33] reported that DM intake of goat was not influenced by the addition of 120g/kgDM Pomegranate Seed Pulp (PSP) in the diet.

The EE intakes of goats were significantly increased (P<0.0001) when the diets supplemented with soybean oil (The SO and PSPSO diets). The ME intakes of the CON and PSP goats were significantly decreased (2.78, 2.73Mcal/d) compared with (P<0.05) the SO and PSPSO goats (3.03, 2.98 Mcal/d). The NDF intakes of goats were significantly increased (P<0.05) when the diets supplemented with pomegranate seed pulp (the PSP and PSPSO diets). The intake of NFC was the lowest in goats receiving PSPSO diet (647.57g/d) and intake NFC was the highest in goats receiving CON diet (723.39 g/d). The intake of NFC was significantly lower (P<0.045) in goats receiving SO diet than PSP diet. Goats with pomegranate seed pulp and soybean oil supplementation (PSPSO diet) had the lowest (P<0.05) NFC:NDF intake (1.04) and CON diet had the highest (P<0.05) NFC:NDF intake (1.23).

Excessive amounts of non-structural carbohydrates intake and inadequate fiber intake may predispose animals to sub-acute ruminal acidosis (SARA) [34]. SARA is digestive disorder in dairy ruminants and affects ruminal fermentation pattern and rumen microbial flora changes and consequently impair ruminants productivity. SARA induction was performed by increasing dietary NFC to NDF ratio from 1.02 to 1.24, 1.63 and 2.58 [35].

In this experiment, dried PSP or SO were used, which resulted differences on the NFC:NDF ratio. In our result the highest NFC:NDF ratio and abundant NFC of CON diet might be considered optimal for minimizing the risk of SARA for dairy goats. Nutrient digestibilities of goat feeds are presented in Table IV. Result similarity to [36], Dietary PSP or SO had no effect on the TTAD of DM (P=0.152), whereas TTAD of EE, NDF and ADF tended to increase (P=0.064, 0.094, 0.072 respectively). The TTAD of CP tended to decrease (P=0.061).

All diets, except CON diet, digestibilities of EE, NDF and ADF tended to increase and digestibility of CP tended to decrease and nevertheless dietary PSP and SO had no effect on the total tract apparent digestibilities of DM, CP, EE, NDF and ADF (P>0.05). Lack of differences in nutrient digestibility was probably due to non-influence of all diets on DMI variation. Moreover the lower TTAD of nutrients were a result of the high lignin content of by-products [37], [38].

The minimum ammonia concentrations require to maximum digestion depended on feed fermentability, if readily degradable carbohydrates were available, higher rumen ammonia N concentration was needed for maximum digestion. The PSPSO diet in this experiment contained a sufficient concentration of available total nonstructural carbohydrates and/or readily degradable carbohydrates (648g/d, NFC intake) and may have required greater ammonia concentration for maximum digestion, which stimulate activity of cellulotytic bacteria and ultimately increased NDF digestibility, compared with the other diets. Increase in NDF and ADF digestions were significantly increased when the diets had higher dietary CP levels. In this experiment dietary CP levels of all rations were high and were iso-proteic averaging 166g/kgDM) and should have digestion maximum NDF (464g/kgDM) digestion and ADF digestion (271g/kgDM) by the PSPSO diet.

TABLE III. INTAKE OF DRY MATTER, NON-FIBROUS CARBOHYDRATE AND NEUTRAL DETERGENT FIBER FROM DIFFERENT EXPERIMENTAL DIETS FED TO LACTATING CROSSBRED SAANEN DAIRY GOATS

Intake	Dietary treatments[1]				SEM[2]	P-value
	CON	PSP	SO	PSPSO		
BW (kg)	46.85	47.88	47.55	47.25	0.86	0.85 [ns]
DMI (g/d)	1652	1624	1558	1594	28	0.15 [ns]
DMI/ $BW^{0.75}$ (g/kg)	92.25	89.22	86.04	88.45	2.25	0.20 [ns]
Protein intake (g/day)	301.45	302.41	301.12	299.96	0.64	0.45 [ns]
EE intake (g/d)	41.10[a]	42.10[ab]	93.95[cd]	92.43[c]	1.10	0.0001
NFC intake (g/d)	723.39[d]	693.42[c]	675.07[b]	647.57[a]	10	0.045
NDF intake (g/d)	589.73[ab]	626.35[cd]	584.02[a]	620.46[c]	5	0.05
[NFC/ NDF] intake	1.23[d]	1.11[b]	1.16[bc]	1.04[a]	0.06	0.05
ME in take(Mcal/d)	2.78[ab]	2.73[a]	3.03[cd]	2.98[c]	0.05	0.05

[1] Values are least-square means.
[2] SEM, standard error of means.
[abcd] means within the same row with the different superscripts differ at P≤0.05 by LSD test.
[NS] not significant (P>0.05).

In this study the total tract apparent digestibility (TTAD) of CP tended (P=0.061) to decrease in PSP, SO and PSPSO diets (Table IV). High concentrations of PSP hydrolysable tannins might reduce proteins digestibility [37] These results indicate that the effects of secondary phytochemicals on proteins/nutrients digestibility vary with phytochemical structure, concentration of these phytocompounds and the geographical cultivar or plant source exploited [38]. The additional amount of digestible EE from PSP, SO and PSPSO diets then may have contributed to decrease TTAD of CP. The reduction amount of digestible CP from PSP and PSPSO diets may be related to high polyphenols content of PSP (42g/kg of DM) or PSP tannins, which reduces CP digestibility by binding directly and by inhibition of proteolytic digestive enzymes in the lower gastrointestinal tract [38] and may limited amino acids absorption. But in this experiment, however, PSP use had no adverse effects of PSP tannins on protein digestibility.

Tendency of increasing digestibility of EE (P=0.064) may partly be associated with an increased intake of dietary EE due to supplementation of soybean oil in the diets (SO and PSPSO diets). Fat intake of SO and PSPSO diets (94 and 92.4g/d) were two-fold higher than that in any of the other groups (41 and 42g/d). Furthermore, fat component of pomegranate by-product in experimental diets are mainly in the pulp and seed (108g/kgDM). PSP lipids and SO lipids contain mainly unsaturated FA, which are shown to be more digestible EE and might be involved in EE intake elevation. Contradiction report, negative effect of limited degree of activity and secretion of pancreatic lipase, co-lipase and bile lipids on absorption of lipids in ruminants, with high fat intake is reduced EE digestibility. Binding of the PSP phytophenolic compounds/tannins with bile salts, cholesterol and cholesterol ester may reduce fat absorption and increase faecal excretion [38]. But in this experiment shows unlimited degree of activity and secretion of pancreatic lipase, co-lipase and bile lipids on absorption of lipids in goat digestive tract.

TABLE IV. TOTAL TRACT APPARENT DIGESTIBILITY (TTAD) OF NUTRIENTS FROM DIFFERENT EXPERIMENTAL DIETS FED TO LACTATING CROSSBRED SAANEN DAIRY GOATS (G/KG DM)

TTAD	Dietary treatments				SEM	P-value
	CON	PSP	SO	PSPSO		
DM	722.09	698.32	704.21	682.93	39.87	0.152 ns
CP	660.56	650.64	643.43	647.67	10.97	0.061 T
EE	643.56	655.64	665.43	710.55	13.87	0.064 T
NDF	440.56	455.64	450.31	463.67	16.08	0.094 T
ADF	250.63	263.64	252.75	270.67	9.02	0.072 T

NS not significant (P>0.05).
T tendency (0.05<P≤0.10).

B. Production Performances of Goats

In Table V are presented the main productive results of goats in the experiment. Raw milk yield was rather high (2.25–2.3kg/day) and milk yield did not differ significantly between dietary treatments. The lack of response in milk yield among the different dietary treatments because animals produced milk and they were switched to a different diet every three weeks. And one of the most important factors in controlling milk yield was DMI, the milk yield increment in the current study (>2.2 kg/day) was partially responsible the maximum goats DMI of all diets (~1.6kg/day–1.7kg/day). Diet palatability might be partially responsible the maximum DMI of goats. High palatability of energy-dense high fat foods or oil inclusion in ration stimulate passive and active consumption and PSPs contain more than 10% crude fat. And in the current study, high CP levels of all diets had slightly enhanced milk production, but highly significant milk protein content and for milk production regulation.

Goats receiving the CON diet had the lowest contents of milk fat (31.2g/kg milk) and protein (29.9g/kg milk). Goats receiving the PSPSO diet had the highest contents of milk fat (34.2g/kg milk) and protein (32.4g/kg milk). Goats receiving the PSPSO diet had the highest yields of average daily 4% FCM (2145g/day), milk fat (80.4g/day) and protein (76.1g/day).

All diets, by the supplementation of pomegranate seed pulp or soybean oil in the experimental diets, did not affect (P>0.10) content of lactose (47.3g/kg–48.3g/kg, P=0.20) and content of total milk solid (119.3g/kg–121.0g/kg, P=0.15), yield of solid not fat (198 g/d–204 g/d, P=0.152) and yield of total solid (268 g/d–284 g/d, P=0.20). Solid not fat content (SCM) tended to decrease (P=0.08). Lactose yield (P=0.064) and solid-corrected yield (SCM yield) tended to increase (P=0.065).

In the current study, however, it seems that the synchronization between N and maximum fiber degradation, NDF and ADF digestibility, is most important factor, which suggested a more efficient utilization of dietary N for milk protein secretion. There was a trend for improve N utilization and was sufficient to allow high milk protein production as a results of the PSPSO diet. Ruminal ammonia content for maximum digestion might have promoted the utilization of metabolizable energy of the PSPSO diet for enhancement of milk fat content (34.2g/kg milk) and yield (80.4g/day) or for fat constituent's in fat biosynthesis process by udder secretory cell of goat lactation than for BW increment. Intake of PSP may have led goats to deposit less body fat and then BW maintain. There is a passage evidence for the channeling of fatty acids. Lipoprotein lipase activity restraint of adipocyte cell helps to channel fatty acids away from adipose tissue into the udder.

The fat to protein ratio lower than 1.05 reflects milk fat depression, a dietary problem causing an energy imbalance, milk fat depression could then contribute to explain the trend for high concentrate level manipulation, abundant non-fibrous carbohydrates intake and inadequate cellulose and hemicellulose intake of CON goats. Goats with CON diet had the highest (P<0.05) NFC to NDF intake (1.23). The NFC to NDF ratio of CON diet for goats might be considered minimal for the risk of SARA.

The highest content (P<0.05) and yield (P<0.05) of milk fat and protein of goats in PSPSO diet may have associated with the appropriate of milk fat to protein ratio, dietary NFC to NFC ratio, NDF intake, NFC intake, EE intake and the NDF and ADF digestibility.

C. Production Efficiency, Somatic Cell Count and Anti-Oxidant Property of Milk

The mean of milk production efficiency, milk fat efficiency and somatic cell count of goat milk are shown in Table VI. Milk production efficiency (milk produced per unit DMI) was significantly influenced (P<0.049) by feeding PSP, SO and PSPSO diets compared with the CON diet.

Milk fat efficiency (fat-corrected milk produced per unit DMI) was significantly influenced (P<0.050) by feeding PSPSO (1.35) diet compared with SO (1.30) diet and SO diet differs from PSP diet and PSP (1.26) diet differs from CON (1.18) diets. The highest efficiency of milk production and milk fat of goats were found in animals offer the dietary PSPSO.

Marginal response to ME intake provides useful information about the biological efficiency of milk fat yield. The average marginal FCM yield response to the CON diet, PSP diet, SO diet, and PSPSO diet allowance were 0.70, 0.75, 0.67, and 0.72 kgFCM per 1 Mcal increase in ME intake (P<0.05). Thus, ME derived from PSPSO in goat diet had tendency in improved nutrients digestibility (EE, NDF, ADF digestibility) throughout each period and had also shown marginal bioefficiency of yields more milk and FCM than ME derived from CON, PSP and SO in goat diets.

TABLE V. PERFORMANCE OF DAIRY GOATS SUPPLEMENTED WITH POMEGRANATE SEED PULP OR SOYBEAN OIL

Performance	Dietary treatments				SEM	P-value
	CON	PSP	SO	PSPSO		
Milk yield (kg/day)	2.25	2.3	2.25	2.3	0.05	0.051^{ns}
Milk component (g/kg)						
Fat	31.2^{a}	32.9^{b}	33.2^{bc}	34.2^{d}	0.04	0.02
Protein	29.9^{a}	30.5^{b}	31.1^{c}	32.4^{d}	0.05	0.04
Lactose	47.5	48.3	45.2	47.3	1.59	0.20^{ns}
Solid-not fat	88.1	87.2	83.5	84.8	0.70	0.08^{T}
Total solid	119.3	120.1	120.7	121.0	0.65	0.15^{ns}
Fat/protein	1.04	1.08	1.07	1.06	-	-
Component yield (g/day)						
4%FCM [1]	1950^{a}	2054^{bc}	2020^{b}	2145^{d}	45	0.001
Fat	70.2^{a}	75.7^{b}	76.4^{bc}	80.4^{d}	0.65	0.045
Protein	67.3^{a}	70.2^{b}	71.53^{bc}	76.1^{d}	0.98	0.024
Lactose	106.9	111.1	106.3	111.2	4.30	0.064^{T}
Solid-not fat	198.2	200.6	201.3	204.0	3.03	0.152^{ns}
SCM [2]	2333	2412	2379	2496	98	0.065^{T}
Total solid	268.4	276.2	277.6	284.4	12.0	0.200^{ns}

[1] FCM yield, Fat-corrected milk yield, FCM yield, g/d = Milk fat corrected in 4%with the equation Y4% = (0.399 × [milk yield (g/day)] + 15.02 × [fat yield (g/day)]).
[2] SCM yield, Solid-corrected milk yield, g/d, SCM yield = (12.3 × Fat yield (g/day)) + (6.56 × SNF yield (g/day)) + (0.0752 × Milk yield (g/day)).
[abcd] means within the same row with the different superscripts differ at P<0.05 by LSD test.
[NS] not significant (P>0.05).
[T] tendency (0.05<P≤0.10).

SCC reduction (P=0.001) goat milk was found in animals offer the PSP and PSPSO diets. The dietary PSP antioxidant polyphenol-rich effect on goats' immune systems stimulation was expected, meaning that animals can be boosted their immunostimulatory by phytophenols &pharmaceuticals properties of dietary PSP. It may have promoted the goats' immunomodulatory response. [8] presented that supplementing polyphenols from pomegranate extracts to pre-weaned dairy calves improved lymphocyte function, which may have improved humoral and cell-mediated immunity.

TABLE VI. MILK QUALITY, MILK YIELD EFFICIENCY AND MILK FAT EFFICIENCY OF DAIRY GOATS SUPPLEMENTED WITH POMEGRANATE SEED PULP OR SOYBEAN OIL

Efficiency	Dietary treatments				SEM	P-value
	CON	PSP	SO	PSPSO		
Milk yield efficiency[1]	1.36^{a}	1.42^{b}	1.44^{bcd}	1.44^{bcd}	0.03	0.049
FCM efficiency[2]	1.18^{a}	1.26^{b}	1.30^{c}	1.35^{d}	0.02	0.050
SCC ×1000 (cells/ml)	825^{c}	597^{a}	845^{cd}	520^{ab}	44	0.01
Marginal FCM efficiency[3] (kg/Mcal)	0.70^{b}	0.75^{d}	0.67^{a}	0.72^{cd}	0.02	0.05

[1] Milk yield efficiency = 4 % FCM (kg/d) / DMI (kg/d).
[2] FCM efficiency = 4 % FCM (kg/d) / DMI (kg/d).
[3] Marginal FCM efficiency = 4 % FCM (kg/d) / ME intake (Mcal/d).
[abcd] means within the same row with the different superscripts differ at P<0.05 by LSD test.

Concentrations of total phenols were not affected (P= 0.10) by all dietary treatments (Table VII). However concentrations of flavonoids were increased (P=0.05) by the PSP and PSPSO diets. These results indicated the PSP and PSPSO diets lead to greater total phenols and total flanoids proportion of goat milk.

Similar to our results, [18] indicated increases in milk antioxidant activity in dairy cows fed concentrated pomegranate extract supplemented diet. Some research has indicated that increases in milk antioxidant activity in dairy goats fed diets containing pomegranate seed oil or linseed oil [16]. [14] found that feeding pomegranate byproduct silage to growing lambs increased antioxidant activity of meat. [17] showed that the increased PSP in the goat kid diet increased total antioxidant activity of muscle.

There is beneficial advantage of PSP for a source of antioxidants, total flanoids or total phenols, to bypass the rumen when animals are fed polyunsaturated fatty acid soybean oil. The highest flanoids/phenolics in milk of goats fed the PSP or PSPSO diets were a result of increased absorption of flanoids/phenolics antioxidant from the gastrointestinal tract and were transferred of these compounds into milk (1.37 mgQE/l, 1.40 mgQE/l), (8.2mgGAE/l, 8.4mgGAE/l) although, these antioxidant compounds were transferred in low milk proportion, but appropriate proportion between antioxidant compounds and those unsaturated FAs may be important [37], thus, the PSPSO and PSP animals were producing the highest oxidative stability milk.

Similarity, Pomegranate rind extract improved the oxidative stability and storage quality of *Kalari* and may be commercially exploited as a natural preservative in cheese products [39]. Regards to Thiobarbituric Reactive Substances (TBARS) as a measure of antioxidant activity, Butyrated Hydroxy Anisole (BHA), synthetic antioxidant, gave lower oxidation values than the natural antioxidants (pomegranate seed extract) when used at the same level in both lipid systems (lard- and canola oil-model) [40].

TABLE VII. MILK ANTI-OXIDATIVE PROPERTY OF DAIRY GOATS SUPPLEMENTED WITH POMEGRANATE SEED PULP OR SOYBEAN OIL

Antioxidant property	Dietary treatments				SEM	P-value
	CON	PSP	SO	PSPSO		
Total phenols (mgGAE[1]/l)	3.8	8.2	4.1	8.4	3.1	0.10 [T]
Total flanoids (mgQE[2]/l)	1.18[a]	1.37[c]	1.19[ab]	1.40[cd]	0.14	0.05

[1] GAE = gallic acid equivalents.
[2] QE = quercetin equivalents.
[abcd] means within the same row with the different superscripts differ at $P<0.05$ by LSD test.
[T] tendency ($0.05 < P \leq 0.10$).

The method to solve the problem of the fraction of pomegranate processing waste, PSP, from mini-scale local enterprise, being rich in moisture and microflora loads, lead directly to environmental pollution. The discarded pomegranate, PSP by-product could potentially be used as a novo food resource for livestock production. Soybean oil inclusion, SO, in the goat ration could be increased milk fat.

The results are showing that use of PSPSO diet for animals increased fat content, fat yield and oxidative stability of goat milk and lead to greater income & profitability.

IV. CONCLUSION

The results of the present study demonstrated that all of diets did not affect goat's body change, DM intake and protein intake. Combination of the dried pomegranate seed pulp at 120g/kgDM and soybean oil at 50g/kgDM (the PSPSO diet) increased intake of crude fat, cell wall and contained a sufficient concentration of available total nonstructural carbohydrates for maximum digestion without negatively affecting goats nutrient digestibility and productivity.

The PSPSO diet increased milk fat yield and content, protein yield and content, and the 4% fat-corrected milk yield of dairy goats. The highest efficiency of milk production and milk fat and milk flanoids antioxidant capacity improvement were found in animals offer the PSPSO diet. The PSPSO diet also decreased somatic cell count of raw milk. And although milk yield efficiency, somatic cell count of milk, milk flanoids of PSP diet did not differ significantly from of PSPSO diet and milk yield efficiency of SO diet did not differ significantly from of PSPSO diet. Only use of pomegranate seed pulp or only soybean oil in diet did not affected for reaching higher nutrient digestibility and goat productivity.

Conclusion, the combination of pomegranate seed pulp and soybean oil (PSPSO) can used in the goat feed for enhancement of goat production. Additionally, our findings show, high quality of milk; flanoids antioxidant; oxidative stability, the highest efficiency of milk yield, milk fat and responsibility of marginal fat-corrected milk yield of goats were found in animals offer the dietary Pomegranate Seed Pulp and Soybean Oil (PSPSO).

ACKNOWLEDGMENT

The author wishes to thank curriculum of animal science, department of animal production technology and fisheries, KMITL for financial support of this work.

REFERENCES

[1] W. Elfalleh, H. Hannachi, N. Tlili, Y. Yahia, N. Nasri, and A. Ferchichi, "Total phenolic contents and antioxidant activities of pomegranate peel, seed, leaf and flower," *Journal of Medicinal Plants Research*, vol. 6, pp. 4724–4730, 2012.

[2] P. Jing, T. Ye, H. Shi, Y. Sheng, M. Slavin, B. Gao, L. Liu, and L. Yu, "Antioxidant properties and phytochemical composition of China-grown pomegranate seeds," *Food Chemistry*, vol. 132, pp. 1457–1464, 2012.

[3] S. Akhtar, T. Ismail, D. Fraternale, and P. Sestili, "Pomegranate peel and peel extracts: Chemistry and food features," *Food Chemistry*, vol. 174, pp. 417-425, 2015.

[4] L. Wang, "Pomegranate juice and pomegranate derived natural products as alternative treatment for cancer progression and metastasis," Ph.D. dissertation, Graduate Program in Biochemistry and Molecular Biology, Univ. of California, Riverside, CA, 2013.

[5] J. Manasathien, "The effects of pomegranate *Punica Granatum* extracts on antioxidation, ultraviolet B, irradiation on rat skin and apoptosis of MCF-7, human breast cancer cells," Ph.D., School of Biology, Suranaree University of Technology, Nakornrachasrima, 2011.

[6] P. Panichayupakaranant, S. Tewtrakul, and S. Yuenyongsawad, "Antibacterial, anti-inflammatory and anti-allergic activities of standardized pomegranate rind extract," *Food Chemistry*, vol. 123, pp. 400–403, 2010.

[7] O. A. Gbinigie, I. J. Onakpoya, and E. A. Spencer, "Evidence for the effectiveness of pomegranate supplementation for blood pressure management is weak: A systematic review of randomized clinical trials," *Nutrition Research*, 2017.

[8] R. A. Oliveira, C. D. Narciso, R. S. Bisinotto, M. C. Perdomo, M. A. Ballou, M. Dreher, and J. E. P. Santos, "Effects of feeding polyphenols from pomegranate extract on health, growth, nutrient digestion, and immunocompetence of calves," *Journal of Dairy Science*, vol. 93, pp. 4280–4291, 2010.

[9] A. H. Ahmed, "The ability of pomegranate to ameliorate symptoms associated with alzheimer's disease in aged transgenic mice," M.S. thesis, Interdisciplinary Neuroscience, Univ. of Rhode Island, RI, 2013.

[10] E. Jami, A. Shabtay, M. Nikbachat, E. Yosef, J. Miron, and I. Mizrahi, "Effects of adding a concentrated pomegranate-residue extract to the ration of lactating cows on in *vivo* digestibility and profile of rumen bacterial population," *Journal of Dairy Science*, vol. 95, pp. 5996–6005, 2012.

[11] J. L. Guil-Guerrero, L. Ramos, C. Moreno, J. C. Zú˜niga-Paredes, M. Carlosama-Y ́epez, and P. Ruales, "Plant-food by-products to improve farm-animal health, review article," *Animal Feed Science and Technology*, vol. 220, pp. 121–135, 2016.

[12] A. Shabtay, H. Eitam, Y. Tadmor, A. Orlov, A. Meir, P. Weinberg, Z. G. Weinberg, Y. Chen, A. Brosh, I. Izhaki, and Z. Kerem, "Nutritive and antioxidative potential of fresh and stored pomegranate industrial byproduct as a novel beef cattle feed," *Journal of Agricultural Food Chemistry*, vol. 56, pp. 10063–10070, 2008.

[13] V. Vasta and G. Luciano, "The effects of dietary consumption of plants secondary compounds on small ruminants' products quality," *Ruminant Research*, vol. 101, pp. 150–159, 2011.

[14] B. Kotsampasi, V. Christodoulou, A. Zotos, M. Liakopoulou-Kyriakides, P. Goulas, K. Petrotos, P. Natas, and V. A. Bampidis, "Effects of dietary pomegranate byproduct silage supplementation on performance, carcass characteristics and meat quality of growing lambs," *Animal Feed Science and Technology*, vol. 197, pp. 92–102, 2014.

[15] A. Emami, M. H. F. Nasri, M. Ganjkhanlou, A. Zali, and L. Rashidi, "Effects of dietary pomegranate seed pulp on oxidative stability of kid meat," *Meat Science*, vol. 104, pp. 14–19, 2015.

[16] A. Emami, M. H. F. Nasri, M. Ganjkhanlou, L. Rashidi, and A. Zali, "Dietary pomegranate seed pulp increases conjugated-linoleic and alpha-linolenic acids in muscle and adipose tissues of kid," *Animal Feed Science and Technology*, vol. 209, pp. 79–89, 2015.

[17] A. Emami, M. Ganjkhanlou, M. H. F. Nasri, A. Zali, and L. Rashidi, "Pomegranate seed pulp as a novel replacement of dietary cereal grains for kids," *Small Ruminant Research*, vol. 123, pp. 238–245. 2015.

[18] A. Shabtay, M. Nikbachat, A. Zenou, E. Yosef, O. Arkin, O. Sneer, A. Shwimmer, A. Yaari, E. Budman, G. Agmon, and J. Miron, "Effects of adding a concentrated pomegranate extract to the ration of lactating cows on performance and udder health parameters," *Animal Feed Science and Technology*, vol. 175, pp. 24–32, 2012.

[19] A. L. M. Marn, P. Gómez-Cortés, A. G. G. Castro, M. Juárez, L. M. P. Alba, M. P. Heránndez, and M. A. D. L. Fuente, "Animal performance and milk fatty acid profile of dairy goats fed diets with different unsaturated plant oils," *Journal of Dairy Science*, vol. 94, pp. 5359–5368, 2011.

[20] M. A. Bouattour, R. Casals, E. Albanell, X. Such, and G. Caja, "Feeding soybean oil to dairy goats increases conjugated linoleic acid in milk," *Journal of Dairy Science*, vol. 91, pp. 2399–2407, 2008.

[21] O. C. Almeida, A. V. Pires, I. Susinb, R. S. Gentil, C. Q. Mendes, M. A. A. Queiroz, E. M. Ferreira, and M. L. Eastridge, "Milk fatty acids profile and arterial blood milk fat precursors concentration of dairy goats fed increasing doses of soybean oil," *Small Ruminant Research*, vol. 114, pp. 152–160, 2013.

[22] E. Tsiplakou and G. Zervas, "Effect of fish and soybean oil inclusion in goat diet on their milk and plasma fatty acid profile," *Livestock Science*, vol. 155, pp. 236–243, 2013.

[23] Bureau of Animal Nutrition Development, Department of Livestock Development, Animal feed and feeding, Thailand. [Online]. Available: www.nutrition.dld.go.th

[24] J. Yaowapaksophon, *Advanced Animal Nutrition*, 1st ed., Bangkok: Amarin Printing and Publishing, 2016, ch. 2, pp. 38-67.

[25] A. Sánchez, D. Slerra, C. Luengo, J. C. Corrales, C. T. Morals, A. Contreras, and C. Gonzalo, "Influence of storage and preservation on fossomatic cell count and composition of goat milk," *Journal of Dairy Science*, vol. 88, pp. 3095–3100, 2005.

[26] Association of Official Analytical Chemist, *Official Methods of Analysis of the Association of Analytical Chemists*, 17th ed., International Gaithersburg, MD, 2000.

[27] P. J. V. Soest, J. B. Robertson, and B. A. Lewis, "Methods for dietary fiber, neutral detergent fiber, and nonstarch polysaccharides in relation to animal nutrition," *Journal of Dairy Science*, vol. 74, pp. 3583–3597, 1991.

[28] A. J. H. V. Es, "Feed evaluation for ruminants. I. The systems in use from May 1977 onwards in the Netherlands," *Livestock Production Science*, vol. 5, pp. 331–345, 1978.

[29] V. L. Singleton and J. L. Rossi, "Colorimetry of total phenolics with phosphomolybdic-phosphotungstic acid reagents," *American Journal of Enology and Viticulture*, vol. 16, pp. 144–158, 1965.

[30] S. A. Zaki, S. H. Abdelatif, N. R. Abdelmohsen, and F. A. Ismail, "Phenolic compounds and antioxidant activities of pomegranate peels," *International Journal of Food Engineering*, vol. 1, pp. 73–76, 2015.

[31] H. P. Makkar, M. Blummel, N. K. Borowy, and K. Becker, "Gravimetric determination of tannins and their correlations with chemical and protein precipitation methods," *Journal of the Science of Food and Agriculture*, vol. 61, pp. 161–165, 1993.

[32] R. G. Woisky, and A. Salatino, "Analysis of propolis: Some parameters and procedures for chemical quality control," *Journal Apicutural Research*, vol. 37, pp. 99–105, 1998.

[33] J. Modaresi, M. H. F. Nasri, L. Rashidi, O. Dayani, and E. Kebreab, "Short communication: Effects of supplementation with pomegranate seed pulp on concentrations of conjugated linoleic acid and punicic acid in goat milk," *Journal of Dairy Science*, vol. 94, pp. 4075-4080, 2011.

[34] J. M. D. Enemark, "The monitoring, prevention and treatment of sub-acute ruminal acidosis (SARA): A review," *The Veterinary Journal*, vol. 176, pp. 32–43, 2008.

[35] D. Liu, X. Zhou, P. Zhao, M. Gao, H. Han, and H. Hu, "Effects of increasing non-fiber carbohydrate to neutral detergent fiber ratio on rumen fermentation and microbiota in goats," *Journal of Integrative Agriculture*, vol. 12, pp. 319–326, 2013.

[36] M. J. Abarghuei, Y. Rouzbehan, A. Z. M. Salem, and M. J. Zamiri, "Nutrient digestion, ruminal fermentation and performance of dairy cows fed pomegranate peel extract," *Livestock Science*, vol. 157, pp. 452–461, 2013.

[37] N. W. Santos, G. T. D. Santos, D. C. Silva-Kazama, P. A. Grande, P. M. Pintro, F. E. deMarchi, C. C. Jobim, and H. V. Petit, "Production, composition and antioxidants in milk of dairy cows fed diets containing soybean oil and grape residue silage," *Livestock Science*, vol. 159, pp. 37–45, 2014.

[38] L. S. Lima, G. T. Santos, A. B. Schogor, J. C. Damasceno, F. E. Marchi, N. W. Santos, F. S. Santos, and H. V. Petit, "Effect of abomasal or ruminal supplementation of citrus pulp and soybean oil on nutrient digestibility and ruminal fermentation of dairy cows," *Animal Feed Science and Technology*, vol. 189, pp. 123–129, 2014.

[39] D. Mahajan, Z. F. Bhat, and S. Kumar, "Pomegranate (*Punica granatum*) rind extract as a novel preservative in cheese," *Food Bioscience*, vol. 12, pp. 47–53, 2015.

[40] C. T. Schevey, "Investigating the efficacy of pomegranate extract as an antioxidant in multiple high-lipid model systems," M.S. thesis, Food Science and Human Nutrition, Univ. of Illinois at Urbana-Champaign, IL, 2012.

J. Yaowapaksophon was born in April 1966 in Chiangmai province, Thailand. She received her Bachelor's and Master's degrees in Kasetsart University, Thailand. She has been a University teacher since 1991. She is currently an assistant professor in animal science at King Mongkut's Institute of Technology Ladkrabang, Bangkok, Thailand. Her teaching area is animal biochemistry, advanced animal nutrition, milk science and technology, and animal biotechnology. She had more than 15 research publications. She had published 2 books and she was coauthor of 1 scientific book published on national. She has been a member of the animal husbandry association of Thailand under the Royal Patronage of H.R.H. Princess Maha Chakri Sirindhorn, the Thai veterinary medical association under the Royal Patronage of His Majesty of the King, and the science society of Thailand under the Patronage of His Majesty of the King. Her main research interests include ruminant nutrition, fatty acid of feed and farm product.