

## Energy balance and biological response of dairy goats fed pomegranate seed pulp and soybean oil

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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 and hydrolysable tannins or soybean oil (SO) as an energy source on energy balance, blood biochemical profile and endocrine change of Saanen goats. Eight lactating cross-bred dairy 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 (CON) no supplementation, supplementation with pomegranate seed pulp (PSP) at 120 g/Kg DM, supplementation with soybean oil (SO) at 50 g/Kg DM, and supplementation with SO at 50 g/Kg DM and PSP at 120 g/Kg DM (PSPSO), of total dry matter intake. Energy balance of the goats did not differ significantly (P>0.05) among different dietary treatments, but body energy balance of the SO or PSPSO goats tended to increase ( $P \leq 0.10$ ) and would better for improving energy balance of the animals. Blood metabolic response of experimental diets, such as glucose, cholesterol, triglyceride, non-esterified fatty acid (NEFA),  $\beta$ -hydroxybutyrate (BHBA) and insulin profile did not change statistically (P>0.05) among different dietary treatments. High-fat diets (SO and PSPSO), however, tended to increase (P≤0.10) glucose, cholesterol, BHBA and insulin and tended to decrease (P≤0.10) triglyceride and NEFA which resulted in an improved blood metabolite for the SO and PSPSO goats. In conclusion, showed a trend of improved energy balance and blood biological response of goats fed the pomegranate by-product and soybean oil.

Keywords: Biological response; Energy balance; Goat; Pomegranate seed pulp; Soybean oil



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## 1. Introduction

The metabolic adaptation capability of lactating animals plays an important role in maintaining energy balance during lactation. Energy balance (EB) is the biological homeostasis of energy in animal, the condition when energy intake is equal to energy expenditure. The EB evaluation can be estimated from the difference between ingested energy (i.e. energy input), and required energy for body maintenance and for the secretion of milk during lactation (i.e. energy output). This EB is highly variable depending on the animal's milk genetic potential, age, lactation stage, health, feed composition and energy profile of the ration [1]. The body EB status of the goats can be estimated by directly measuring the body composition. Some information used computer tomography measurements on lactating goats to predict the changes in body composition [2]. Also, animal's energy status can be estimated by changes in blood metabolic profile [3]. Blood biochemical profile, such as glucose, BHBA, NEFA, cholesterol, triacylglycerol, and hormonal profile, such as insulin, may lead to apathological metabolic modifications (e.g. increase of gluconeogenesis, lipid and protein metabolism), and the two profiles may serve as candidate mediators for goat's energy status. Blood metabolite and hormone in

goats reflects a great sophistication of metabolic control in experimental animals. Normal physiology and normal range of blood metabolic parameters can be confirmed by the absence of morphological evidence and clinical signs of disease in goats. The parameters can also indicate a healthy status of between-animal and sufficient energy intake of evaluation-feed animal.

In Thailand, plant-food byproducts from the food processing industry, small holding agriculture, household sector and fruit juice venders have a substantial potential value as animal feedstuffs that could support animal production and improve farm-animal health [4]. Pomegranate seeds pulp (PSP) is a byproduct derived pomegranates juice. PSP as livestock feeds can be a valuable source to provide dietary nutrient and energy for growth and milk production and reduce the cost of animal production. At present, research on the effect of pomegranate byproducts is still qualified for ruminant nutrition, such as pomegranate seed pulp in goat kids [5], pomegranate seed pulp in dairy goats [6], pomegranate seed pulp in Holstein heifers [7], pomegranate silages in growing lambs [8], concentrated pomegranate extract in lactating cows [9], pomegranate peel extract in dairy cows [10].

Pomegranate seeds pulp (PSP) byproduct feedstuffs and/or Pomegranate (*Punica granatum* L.) have exerted beneficial advantages, are attractive source of bioactive compounds and natural food preservatives. Pomegranate medicinal bioactive ingredients include several groups of phytochemical compounds [11], in particular, phenolic compounds which have high correlation with disease-fighting compounds and help to maintain human & animal health to preventing diseases. As alternative feed resource, the pomegranate by-products could be used as new raw material of dietary nutrient and energy in support of milk production and improve animal health. Nonprevious research reported energy status of goats fed PSP. Thus, PSP's potential was encouraged the formation of this trial on value-addition capacity of feedstock PSP waste.

Supplemental lipids in feed formulations are used to increase the rations' energy density and enhance nutritional and economic strategy, and recent studies have found that soybean oil (SO) may have the potential to be a good source of energy for livestock nutrition.

Dietary SO manipulation could develop the nutritional and energy quality of goat milk to increases proportions of monounsaturated fatty acids and polyunsaturated fatty acids in milk fat, thereby improving health of human consumers [12-14]. However, nowadays, to author acknowledge, no studies have focused on the combined effects of these powerful ingredients (PSP, SO) on key plasma metabolites and metabolic hormones that influence energy use in lactating dairy goats. Thus, the objective of this experiment was to investigate the effects of supplementing two feedstuffs, dried pomegranate seed pulp (PSP) or soybean oil (SO) to improve blood metabolic profile and energy balance of goats.

#### 2. Materials and Methods

# 2.1 Animals, diets, gross chemical analysis of feeds, facilities, managements, experimental design, and statistical analyses:

The protocol used in this study was provided in [15-16]. The four dietary treatments were designated as follow: 1) control diet/ no supplementation (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. The proportion of the ingredients of the diets and nutrient profile of experimental diets are presented in Table I. Goats were exposed to each 4 treatments using replicated 4X4 Williams Latin square design. 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). Animals were monitored for energy

balance only in the third period of the experiment. Plasma samples were collected from goats for biological response in every sample collection periods.

Table 1. Ingredients o	f concentrate diets, nutrient	profile of	experimental diets,	fat and	metabolisable e	energy fed to the g	goats.
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	Dietary treatments <sup>1</sup>					
Ingredients of concentrate diets (g/KgDM)	CON	PSP	SO	PSPSO		
Maize, ground	248	245	358	338		
Broken rice	208	185	208	206		
Fine rice bran	100	0	100	0		
Bone meal	18	18	18	18		
Soybean meal	400	406	290	292		
Pomegranate seed pulp (PSP) <sup>2</sup>	0	120	0	120		
Soybean oil (SO)	0	0	50	50		
NaCl	18	18	18	18		
Mineral vitamin mix	8	8	8	8		
Nutrient profile of experimental diets (g/KgD	<b>M</b> ) <sup>3</sup>					
СР	166.39	166.93	140.84	140.37		
EE	24.35	23.47	26.29	25.16		
ash	56.05	52.33	52.74	49.76		
NDF	326.30	346.51	325.32	345.10		
ADF	184.89	203.02	181.30	199.25		
NFC	396.81	380.40	426.43	411.10		
Total phenols	3.50	4.20	3.50	4.20		
Total tannins	1.00	3.95	1.00	3.90		
Condensed tannins	0.90	1.50	0.90	1.50		
Hydrolysable tannins	0.10	2.45	0.10	2.40		
ME (Mcal/KgDM)	2.43	2.40	2.49	2.46		

<sup>1</sup>Refer to the control diet (CON), 120 g/kgDM of pomegranate seeds pulp (PSP) added to control diet, 50 g/kgDM of soybean oil (SO) added to control diet, and 120 g/kgDM of pomegranate seeds pulp+50 g/kgDM of soybean oil (PSPSO) added to control diet. <sup>2</sup>The CP, EE, NDF, ADF, total phenols, total tannins of PSP were 113, 108, 440, 320, 42 and 33 (g/kg of DM) respectively. <sup>3</sup>Base on the dietary treatments consisted of Finger hay and concentrates with a forage/concentrate=36/64.

#### 2.2 Blood sampling and analyses:

Blood samples were taken into tube containing heparin anticoagulant. The blood was centrifuged at 2,500 × g for 20 min at 4°C and the plasma was frozen (-20°C) until plasma analysis. Plasma glucose was enzymatically determined by direct reading of the glucose oxidase/peroxidase enzyme method using the Trinder reagent procedure as described by [17]. Plasma triglyceride concentration was quantified enzymatically using cholesterol esterase and glycerol phosphate oxidase after hydrolysis by lipoprotein lipase using paired the reaction with the classic Trinder reaction as described by [18]. Plasma cholesterol was determined by the method of Allain as described by [19]. Plasma NEFA levels were determined using the Acetyl-CoA oxidase method. Plasma BHBA concentration was measured by the enzymatic oxidation of BHBA to acetoacetate by the enzyme BHBA dehydrogenase followed by determination of NADH as described by [20]. The absorbance values for the metabolites were read at specific wavelengths using a Shimadzu (Model UV-3401 PC) UV-Vis Recording Spectrophotometer (Shimadzu Scientific

Instruments, Inc.,). Concentrations of plasma insulin were determined by double-antibody RIA [21].

#### 2.3 Calculation of energy values and energy balance of the goats:

Energy balance was calculated as ME intake less ME for maintenance and milk production according to the equation; EB (Mcal/d) = ME intake (Mcal/d) – ME milk (Mcal/d) – ME maintenance (Mcal/d), where ME intake was calculated according to the equation; ME intake (Mcal/d) = ME content of diet (Mcal/kgDM) × DM Intake (kg/d), ME requirements for milk production (ME milk) was estimated from the net energy (NE) in milk correcting for the efficiency of use of ME intake for production. ME milk = NE milk/0.589. NE for milk production was obtained from the equation; NE milk (Mcal/d) = milk yield (kg/d)/10 × [1.4694 + (0.4025 × milk fat %)], and ME requirements for maintenance (Mcal/d) were assumed to be 110 Kcal × BW<sup>0.75</sup>.

#### 3. Results

#### 3.1 Energy of the goats

In the third period, on the beginning day of experiment and the day of sample collection, ME for maintenance and for milk production and EB of the goats did not differ significantly (P>0.05) among different dietary treatments. When compared with the beginning day, all feeds tended to increase (P $\leq$ 0.10) EB of the goats on the day of sample collection. Negative EB of animal was explained by a marginally significant reduction (P $\leq$  0.10) in body energy position at the collection day for CON and PSP goats (-0.03, -0.01 Mcal/d respectively) and a marginally significant reduction (P $\leq$ 0.10, - 0.11, -0.01 Mcal/d respectively) (Table 2, Fig 1). Positive EB of animal was explained by a marginally significant increasing(P $\leq$ 0.10) in body energy position at the collection day (the last day of feeding) for SO and PSPSO goats (0.14, 0.09 Mcal/d respectively) and a marginally significant enhancement (P $\leq$ 0.10) (or tended to increase EB) in body energy position at beginning

Matabalianananatana		Dietary	CEN/	D == =1		
Metabolic parameters	CON	PSP	SO	PSPSO	SEM <sup>6</sup>	P-value
ME intake, Mcal/d	2.78 <sup>ab</sup>	2.73ª	3.03 <sup>cd</sup>	2.98c	0.05	<u>&lt;</u> 0.05
The first day of period (Mcal/d)						
ME maintenance <sup>3,4</sup>	1.88	1.83	1.94	1.93	0.04	<u>≤</u> 0.10 <sup>т</sup>
ME milk <sup>5</sup>	1.00	1.01	1.03	1.06	0.015	<u>≤</u> 0.10 <sup>т</sup>
The collection period (Mcal/d)						
ME maintenance <sup>3,4</sup>	1.84	1.85	1.86	1.85	0.005	<u>≤</u> 0.10 <sup>т</sup>
ME milk <sup>5</sup>	1.00	1.02	1.03	1.04	0.01	<u>&lt;</u> 0.10 <sup>™</sup>

 Table 2. Energy partitioning<sup>2</sup> of the goats fed pomegranate seed pulp or soybean oil. Goats were monitored for energy balance in the third period of the experiment.

<sup>1</sup> Refer to the control diet (CON), 120 g/kgDM of pomegranate seeds pulp (PSP) added to control diet, 50 g/kgDM of soybean oil (SO) added to control diet, and 120 g/kgDM of pomegranate seeds pulp+50 g/kgDM of soybean oil (PSPSO) added to control diet. <sup>2</sup> Energy partitioning (Mcal/day) for balance = ME intake (Mcal/day) – ME milk (Mcal/day) – ME maintenance (Mcal/d).

<sup>3</sup>ME requirements for maintenance (Mcal/d) were assumed to be 110 Kcal × BW<sup>0.75</sup>.

<sup>4</sup> Metabolic probing revealed that all goats were in dilution maintenance effect.

<sup>5</sup> ME milk = NE milk/0.589. NE for milk production was obtained from the equation; NE milk (Mcal/d) = milk yield  $(kg/d)/10 \times$ 

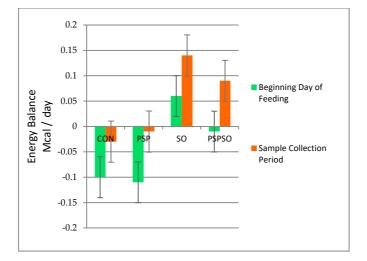
[1.4694 + (0.4025 × milk fat %)].

<sup>6</sup> SEM=standard error of means.

<sup>abcd</sup> Means within the same row with the different superscripts differ at P<0.05 by LSD test.

<sup>T</sup> Tendency ( $0.05 < P \le 0.10$ ).

day for SO goats (0.06 Mcal/d). Or compared with the CON and PSP diets, the SO or PSPSO diets would be better for improving energy balance. Thus, soybean manipulation in livestock nutrition induced a trend of enhance energy balance of the goats.



**Figure 1.** Effects of pomegranate seed pulp (PSP) or soybean oil (SO) and combination of these two feedstuffs (PSP/PSPSO) in diets on goat's energy balance at the beginning day of feeding and the day of sample collection. Animals were monitored for energy balance only in the third period of the experiment. Energy Balance (Mcal/d) = ME intake – ME milk – ME maintenance. Energy balance at the beginning day (green bar); energy balance at the last day of feeding (orange bar).

Matahalia menematana		Dietary tı	CEN (4	D 1		
Metabolic parameters	CON	PSP	SO	PSPSO	SEM <sup>4</sup>	P-value
Nutritional profile						
Glucose (mg/dL)	67.2	65.5	70.0	68.7	0.97	<u>≤</u> 0.10 <sup>™</sup>
Cholesterol (mg/dL)	82.67	93.28	101.96	124.45	8.88	<u>≤</u> 0.10 <sup>™</sup>
Triglyceride (mg/dL)	86.46	84.37	72.45	70.69	4.04	<u>≤</u> 0.10 <sup>™</sup>
NEFA <sup>2</sup> (mEq/L)	0.39	0.44	0.28	0.34	0.03	<u>≤</u> 0.10 <sup>™</sup>
BHBA <sup>3</sup> (mMol/L)	0.46	0.38	0.84	0.75	0.11	<u>&lt;</u> 0.10 <sup>™</sup>
Metabolic hormone						
Insulin (µU/mL)	15.1	14.8	18.3	17.4	0.86	<u>&lt;</u> 0.10 <sup>™</sup>

**Table 3.** Blood metabolic profile of the goats fed pomegranate seed pulp or soybean oil.

<sup>1</sup>Refer to the control diet (CON), 120 g/kgDM of pomegranate seeds pulp (PSP) added to control diet, 50 g/kgDM of soybean oil (SO) added to control diet, and 120 g/kgDM of pomegranate seeds pulp+50 g/kgDM of soybean oil (PSPSO) added to control diet. <sup>2</sup>NEFA= non-esterified fatty acid.

<sup>3</sup>BHBA=β-hydroxybutyrate.

<sup>4</sup>SEM=standard error of means.

<sup>T</sup>Tendency (0.05<P<u><</u>0.10).

#### 3.2 Blood Metabolic Profile (Glucose Cholesterol Triglyceride NEFA BHBA)

Average metabolite and metabolic hormone concentrations are presented in Table 3. Although plasma glucose, cholesterol, triglyceride, non-esterified fatty acid (NEFA),  $\beta$ -hydroxybutyrate (BHBA) and insulin concentrations did not change statistically (P>0.05) in the current study, but high-fat diets (SO, PSPSO) tended to increase (P $\leq$ 0.10) glucose, cholesterol, BHBA and insulin and tended to decrease (P $\leq$ 0.10) triglyceride and NEFA. Blood data for each parameter resulted within the normal range which reflects better nutritional status and health of the experimental animals, confirmed by the absence of any clinical signs of disease.

#### 4. Discussion

#### 4.1 Energy status of the goats

ME intake derived from soybean oil supplementation of high-fat diets (SO, PSPSO diets) (3.03, 2.98 Mcal/d respectively) had improved body energy position of goats throughout the third period of the experiment. Thus, the SO and PSPSO goats were in positive EB. ME intake derived from CON diets (2.78 Mcal/d) had improved body energy position (0.07 Mcal/d) of goats to set nearly net/zero EB (-0.03 Mcal/d). ME intake derived from PSP diets were able to improve body EB (0.10 Mcal/d) of goats to net/zero EB (-0.01 Mcal/d) throughout the entire period. Thus, the CON and PSP goats were in slightly negative EB because animals were almost sufficient when ME intake. The reasons for adequate ME intake might be ration quality & quantity and amount of feed intake [15]. The high energy intake of the goats fed the highest energy rations provided a high supply of substrates from ruminal digestion which supported high milk fat secretion [2].

According to our initial assumption, 50 g/Kg of SO supplement, unsaturated fatty acid of plant fat, in both SO, PSPSO diets were expected to increase ME intake around 9–10%. This incremental ME intake considerably prevented negative energy status of the goats. Thus, the PSP goats sustained BW or PSP diet had no effect on BW change. This agrees with previous EB studies in dairy goats showed that feeding fatty acid (rumen unprotected CLA methyl ester) via concentrate feed improved calculated EB in dairy goats after 14 days of fatty acid supplementation and with no changes in BW [22]. Improved energy status, bioenergetic variables and increased milk yield was also observed with fatty acid (trans-10, cis-12 CLA) plus ad libitum feeding of a total mixed ration in dairy cows [23]. In the present study, all experimental diets had no effect on BW change, however the short periods make observation of changes in BW difficult, the CON and PSP goats were slightly poor in EB, nevertheless, the energy in the diets had sufficient to meet requirement for body maintain and milk production according to the recommendation of Thailand DLD [24]. Experimental diets did not affect (P>0.05) the variation of the goat's BW, DM intake throughout every period of lactation [15]. This study showed that all goats were in reasonable body condition and dilution maintenance. Apportionment of maintenance energy of farm animals, energy for service functions were 45% for kidney work, heart work, respiratory, nervous function and liver function and energy for cell maintenances were 55% for ion transport, protein, and fat turnover [25]. In the current study goats may have already expressed their maximum genetic potential for milk production, and therefore portioned energy spared from ME intake towards milk synthesis rather than improving EB. Animal's energy status / change in body energy reserve / energy deficiency was the main reason of changes in blood concentration of metabolite [26].

#### 4.2 Glucose

No differences (P>0.05) were observed in concentrations of plasma glucose with plant oil and the PSPs by-product supplementation in goat feeds in the current study, although plasma concentration of glucose tended (P $\leq$ 0.10) to be higher in goats fed high fat content diets (SO/PSPSO). Plasma glucose level, the indicator of normal physiology remained similar in all experimental diets and within the normal range and in agreement with previous studies in dairy goats [6, 12, 22, 27]. This is also consistent with results reported by [28] in which no differences were observed in concentrations of blood glucose when growing dairy goats were fed 3 and 15% poultry fat. Contrary, concentrations of blood glucose have been shown to increase as lactating goats were fed diets with supplementation of safflower oil at level of 50 g/kg of the TMR and linseed oil at level of 50 g/kg of the TMR [29]. It was anticipated that fat & oil inclusion did not changed in rumen fermentation and had not decreased glucogenic nutrient availability. All diets in the cur-

rent study were associated with a shift in rumen fermentation towards propionate at the expense of acetate, consistent with responses to sunflower oil reported in lactating cows [30]. Soybean oil (SO) and sunflower oil are in proportion or same as fatty acid type (rich in C18:2 n-6) because they are composed of oleic acid (omega 9) 23% and 14–40%, respectively, and linoleic acid (omega 6) 51% and 48–74%, respectively [31], these are blends of oil benefits, and use of high concentrate (F/C =36/64) [15,16], gave the propionate as fermentation results. Thus, all diets provided additional propionate to the liver as glucone-ogenic and anaplerotic precursors, the propionate would have an anaplerotic effect [31] in regard to tricarboxylic acid (TCA) cycle intermediates in the liver, this propionate fraction was converted to glucose in the liver, tended to improve plasma glucose profile and energy performance.

#### 4.3. Cholesterol and triglyceride

The plasma cholesterol and triglyceride concentrations were not influenced (P>0.05) by feeding SO and the PSPs by-products and were within the normal range indicating a good nutritional and health status of the experimental animals in the current study and in agreement with previous studies in dairy goats fed 2.5 g/kg pomegranate seed oil or 2.5 g/kg linseed oil [6] but contrary to previous findings [27,28]. Contrary, addition of 37 g/kg DM of sunflower oil in lactating goats increased plasma cholesterol concentration [27] and inclusion of 50 g/kg safflower oil or 50 g/kg linseed of the TMR increased plasma triglyceride in lactating goats [29]. In the current study, however, the high-fat diets (SO, PSPSO) tended ( $P \leq 0.10$ ) to decrease plasma triglyceride or the tended to increase plasma cholesterol ( $P \leq 0.10$ ). We hypothesized that a low flow of plasma triglyceride or high flow of plasma cholesterol of the high- fat diets (SO, PSPSO). The higher concentration of triglyceride adaptive profile (84.37–86.46 mg/dL) was related to adaptability of animal fed lower ME intake of the PSP (2.73 Mcal/d) and CON goats (2.78 Mcal/d) [15]. The level of goat's triglyceride was low due to circulating triglyceride to mammary secretary cells and its uptake and increase triglyceride supply for milk and milk fat production. The anabolic activity of adipose tissue usually is high when animals return to a positive EB and/or zero EB [31]. And at metabolic steady state of energy of the PSPSO and SO animals when metabolic flux of anabolic and catabolic processes are going at the same rate. This leads to the development of a steady state for maintaining the animal EB which the concentrations of the intermediates remain constant in the PSPSO and SO goats which derive from their rates of energy formation have come to be in exact balance with their rate of energy expenditure or energy turnover occurrence.

## 4.4. Non-esterified fatty acid (NEFA)

The lack of difference (P>0.05) in the plasma NEFA level between our goats fed the control and goats fed pomegranate seed pulp and soybean oil and was in accordance with other studies with goats [28,29]. The safflower oil at level of 50 g/kg of the TMR and linseed oil at level of 50 g/kg of the TMR did not affect plasma NEFA of the lactating goats [29]. [28] did not see any effect on NEFA level in response to 3% and 15% poultry fat supplementation of growing dairy goat wethers. However, our NEFA results may be in contrast with previous studies [22] which proposed that adding fatty acid (rumen unprotected CLA methyl ester) mixed to concentrate of goat diet might decrease NEFA of the lactating goats. As blood NEFA level may serve as indicators for goat's energy status, If goats were reported as a source of energy for underfed status or in negative energy balance, the effect of stress situation on animals, and goats after transportation, there was a high rate of lipolysis in adipose tissue which elevated blood NEFA concentrations. Moreover, the higher concentration of metabolites like NEFA was related to nutrition for animals adapted to lower forage quality or high fiber ration. While these data indicate that goats consuming fat supplementation diets were in a better energy status also showed lower NEFA levels than their counterparts. For an explanation, the circulating NEFA level was closely linked to the whole-animal EB, SO and PSPSO goats had  $\sim 20 - 30\%$  reduction in blood NEFA which tended to substantiate the improved calculated EB by 0.08 - 0.10 Mcal/d when compared to CON and PSP goats. In actual fact, the normal range of NEFA in the plasma of all animals associated with the unaffected levels of milk yield of our study might indicate a normal physiological function of the experimental animals, which sustained milk production and body energy balance.

#### 4.5. β-hydroxybutyrate (BHBA)

There were no between-diet differences (P>0.05) in plasma  $\beta$ -hydroxybutyrate (BHBA) adaptive profile of animal. These results agree favorably with findings of [12] in which the arterial BHBA concentration (1.19±0.18mMol/L) of the dairy goat was not influenced by the inclusion of increasing doses of soybean oil at the level of 30, 60 or 90 g/d. The invariability of average BHBA concentration, probably, because the animals were receiving diets that met their energy requirements, since energy deficiency was the main reason of changes in blood concentration of this BHBA/NEFA. BHBA concentrations were always greater NEFA or equal NEFA concentrations or the at least twice the amount of BHBA as NEFA. Therefore, NEFA concentrations were lower, rapidly cleared and/or transformed to BHBA. Consequently, fat supplement for animals (SO, PSPSO) tended to increase (P≤0.10) plasma BHBA and we also found a negative correlation between blood BHBA and NEFA adaptive profile of animal in the current study.

#### 4.6. Insulin

No differences (P>0.05) occurred in concentrations of insulin among experimental diets and all goats could maintain nutrient availability to sustain synthesis of milk and milk components in lactating dairy goats. However, plasma insulin tended ( $P \leq 0.10$ ) to be higher in high-fat diets in the current study. Blood insulin of the multiparous goats showed a trend of declining ( $P \le 0.10$ ) during use of the PSP and CON diets and showed a trend of increasing (P $\leq$ 0.10) during use of the high fat content diets. This suggests that the enhancement of protein digestibility and NFC intake and the diminution of ME intake and EE intake of the PSP and CON goats [15] lowered the stimulatory effect of circulating insulin secretion (14.8, 15.1  $\mu$ U/mL respectively). Insulin, a potent lipolytic inhibitor, boosts lipogenesis of fatty tissue and mammary gland [32]. Moreover, insulin activates protein synthesis and glucose utilization in insulin-sensitive tissues of animal (e.g., adipose and skeletal muscle) [33]. The multiparous PSPSO and SO goats had greater insulin concentrations that resulted in a ~20 - 30% decrease in circulating FFA levels compared with the multiparous PSP and CON goats. Increased insulin likely inhibited lipolysis, causing a reduction in plasma FFA concentrations. Furthermore, insulin inhibits complete oxidation of fatty acids, which increases production of ketone bodies [31]. In the present study, greater insulin secretion observed in the PSPSO and SO diets may have inhibited complete oxidation of fatty acids of multiparous goats, because concentrations of BHBA adaptive profiles increased by  $\sim$ 45 – 60% compared with the PSP and CON diets.

### 5. Conclusions

Energy balance and blood biological response exhibited a tendency to improve during use of high fat content diets (SO, PSPSO) diets and therefore, manipulation strategy of goat feed using pomegranate seeds pulp and soybean oil (SO, PSPSO) may be helpful to increase productivity of the goats and health promotion and may be used as a value-addition feed resource and a nutritional tool to alleviate feed shortage.

Supplementary Materials: The following are available online at <a href="https://drive.google.com/file/d/1xbVJoP6-Oe5cbNST6AN\_8smbHGjkLdXw/view?usp=sharing">https://drive.google.com/file/d/1xbVJoP6-Oe5cbNST6AN\_8smbHGjkLdXw/view?usp=sharing</a>

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