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Potential role of dietary pomegranate seed powder (PSP) (*Punica granatum* L.) and / or *Saccharomyces cerevisiae* (SC) on lipid profile of Local male lambs

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Abstract

This study was conducted to find out the effect of adding pomegranate seeds (PSP) and Saccharomyces cerevisiae (SC) to the diet of local lambs on their lipid profiles. (Total Cholesterol (TC), Triglyceride (TG), High Density Lipoprotein (HDL-C), Low Density Lipoprotein (LDL-C), and Very Low Density Lipoprotein (vLDL-C)) It was implemented in the animal field of Baghdad University's veterinary medicine college and ran from 12/1/2022 to 12/6/2022, with 20 lambs aged 2-3 months divided into four groups, with G1 serving as the control group fed concentration diets. G2 fed the same ration and 3% dried pomegranate seed pomace powder (PSP), G3 fed the same ration and 4% PSP with 3g of Saccharomyces cerevisiae (SC), and G4 fed the same ration and 6% PSP with 3g of *Saccharomyces cerevisiae* (SC), water supply at Libdum . Samples of blood were taken from all lambs monthly to measure some biochemical characteristics, including total cholesterol (TC), triglycerides (TG), low-density lipoprotein (LDL-C), high-density lipoprotein (HDL-C), and very-low-density lipoprotein (VLDL-C) statistically significant differences ($P < 0.05$) were discovered in the results of The administration of PSP and SC with diets revealed a remarkable effect between treated and untreated groups for lipid profile aspects in some periods of the experiment when compared to the control group, so it could be concluded from this study that using PSP and/or SC to treat groups could contribute to improving lipid profile traits in male lambs.

Keywords: pomegranate seeds, saccharomyces cerevisiae.

Introduction

When ruminant feeding is based on quantity rather than quality, ruminant feeding is inadequate for a long period of time, resulting in a large gap between the animal's needs and feed availability. As a result, it is generally established that every attempt to boost their production must pass through the improvement of their feeding system. When it comes to regulated diets that provide nutrition for the microbes in the rumen and the growth of the animal, nutritionists are focusing on feed characterization based on their chemical makeup and the composition of their various pieces [1], Ruminants,

in particular, have a remarkable ability to consume fiber due to their rumen microbes. This means that these by-products can essentially replace grains [2] ,As a result, nutritionists and microbiologists have focused their efforts on improving feed consumption and ruminant production efficiency by manipulating the rumen microbial community. Dietary addition and probiotics (bacterial and yeast cultures) are primarily employed to increase rumen activity, and feed efficiency. They are feed supplements that have been used to enhance synthetic chemical feed supplements and antibiotics as growth and development promoters [3].

A lipid profile can be determined by a blood test that can measure the amount of total cholesterol (TC), low-density lipoprotein (LDL), the "bad cholesterol," high-density lipoprotein (HDL), the "good cholesterol," and triglycerides (TG), the most common type of fat in your body in animal blood [4].

One of the fruit by-products used in animal feed is pomegranate (Punica granatum L.), which has gained popularity in recent years due to its nutritional and medical benefits, as well as its attractive appearance. It is also used for cosmetic and pharmaceutical applications due to its medicinal benefits [5].

Pomegranates are high in antioxidants such as anthocyanins and tannins, which may help prevent cholesterol buildup in arteries and hence protect the heart This fruit's juice may help lower the body's concentration of low-density lipoproteins, which could protect the body from stroke attacks, as well as Pomegranate leaf extract has the potential to lower serum cholesterol in mice fed a high fat diet. For a 5-week period, 400 to 800 mg of the extract were given. The rats were fed a high-fat diet and showed signs of obesity. Following this induction, the body's cholesterol and triglyceride levels were significantly reduced. The effects of pomegranate leaf extract on energy and HDL levels in the body were shown to be positive [6].

Jandal [7] indicated a decrease in the levels of cholesterol and triglycerides, LDL, vLDL, and increased HDL levels in the blood when feeding rats on a diet containing pomegranate peel at rates of 1.5%, 3%, and 4.5%., On the other hand, the concentration of total blood cholesterol in fat-tailed lambs decreased when the pomegranate by-product was added to the diet by 5%[8]. It was found that feeding rats on pomegranate peel with 2.5% added to the diet led to a decrease in blood cholesterol and triglycerides [9]

Recent researches on the effects of Saccharomyces cerevisiae (SC) as feed additives has sparked interest [10, 11, 12], but previous and recent studies have produced conflicting results.These discrepancies can be attributed to a variety of factors, including feeding levels [13], protein degradation and levels [14], and the ratio of concentrate to forage [15]. the nutrient composition of the food and the quality of the forage, the quantity of yeast added, and the kind and amount of viable yeast [16].

Saccharomyces cerevisiae (SC) increases propionic acid production, which is the primary substrate for glucose synthesis in ruminants, according to these researchers. Increased mannan-oligosaccharide (MOS) concentrations, which are VFA substrates and promote an increase in the energy metabolism parameters, could explain the

higher BG level in sheep fed with dried yeast. Additionally, yeast supplementation improved iron salt absorption from the small intestine, positively impacting hemoglobin (Hb) formation processes[17].

As a result, the goal of this study was to find out how dietary pomegranate seed powder mixed with Saccharomyces cerevisiae affected the lipid profile in local male lamb**.**

Materials and Methods

This experiment was carried out in the animal farm of the University of Baghdad's College of Veterinary Medicine. The experiment lasted from January 12, 2022, through June 12, 2022. A reputable and well-known source provided twenty local-breed male lambs weighing 17.50 kg and aged 2 to 3 months. Lambs were placed into four groups, taking into account the live weight of lambs each with a single pen measure 2.5 x 4 m. All pens were furnished with cans to use for concentrate and forage diets at the same farm. Before the experiment began, clean, fresh water was always available.

Animal feeding

1-The first group (G1), which is the control group, was fed on concentrated fodder at a rate of 2.5% of body weight with wheat straw and fresh alfalfa.

2-The second group (G2) was fed on the same ration as the first group, and 3% of the dried and crushed pomegranate seeds pomace powder (PSPP) were added to it with the concentrated ration.

3-The third group (G3) was fed on the same diet as the control group with the addition of 4% of the dried and crushed pomegranate seeds pomace powder (PSPP) and 3 gm of bread saccharomyces cerevisiae (SC) per head daily with the concentrated diet.

4-The fourth group (G4) fed on the ration of the control group, in addition to 6% of dried and crushed pomegranate seeds pomace powder (PSPP) and 3 gm of saccharomyces cerevisiae (SC) bread yeast for each head daily added to it with the concentrated ration, With the concentrated ration.

The PSP were obtained at a local market as aby-product, and SC as Baker's yeast powder are used.

Blood samples were drawn from all lambs monthly to measure some biochemical characteristics, including Total cholesterol (TC), triglycerides (TG), low-density lipoprotein (LDL-C), high-density lipoprotein (HDL-C), very-low-density lipoprotein (VLDL-C).

Determination of serum Total Cholesterol concentration mg/dl.

Total cholesterol (TC)concentration was conducted according to Flegg (1973) is the first to use "cholesterol dehydrogenase" to measure in saponified serum extracts, [18,19] have previously discovered a cholesterol ester hydrolase that was capable of converting cholesterol esters to free cholesterol. Finally [20], were able to mix the es-

terase and oxidase into a single enzymatic reagent for the detection of total cholesterol; by using spectrophotometric method [21].

Determination of Triglyceride (TG) mg/dl

This triglyceride(TG) technique is based on a set of intertwined enzymatic reactions [22]. A combination of bacterial lipases digested the triglycerides in the sample, yielding glycerol and fatty acids. Adenosine triphosphate (ATP) phosphorylates glycerol in the presence of glycerol kinase (GK) to create glycerol-3-phosphate. In the presence of GPO (glycerol phosphate oxidase), glycerol-3-phosphate was oxidized by molecular oxygen to create hydrogen peroxide (H2O2) and dihydroxyacetone phosphate. In the presence of peroxidase (POD), the generated H2O2 combines with 4-aminophenazone and N, N-bis (4-sulfobutyl)-3,5-dimethylaniline, disodium salt (MADB) to produce a chromophore that was read at 660-800nm.The sample's triglyceride concentration is proportional to the rise in absorbance at 660–800 nm[23].

Determination of High density lipoprotein (HDL-C) cholesterol concentration mg/dl

The HDL-Cholesterol assay is a two-reagent homogeneous method for measuring HDL-Cholesterol in the context of other lipoprotein particles in serum or plasma. Two phases make up the assay. In the first step, non-HDL-lipoproteins' free cholesterol is solubilized and eaten by cholesterol oxidase, peroxidase, and (4-sulfobutyl)-m-toluidine disodium (DSBmT), resulting in a colorless end product. A special detergent solubilizes HDL-lipoproteins selectively in phase two. The HDL cholesterol was liberated for reactions with cholesterol esterase, cholesterol oxidase, and a chromogen system, resulting in a blue color complex that could be detected bichromatically at 600 and 700 nm. It was found that the rise in absorbance is related to the amount of HDL-C in the sample[24].

Low density lipoprotein (LDL-C) cholesterol concentration mg/dl

The LDL-Cholesterol assay was a homogeneous two-reagent system. There were two unique steps to the test. In the first phase, a special detergent is used to dissolve cholesterol from non-LDL lipoprotein particles. Cholesterase, cholesterol oxidase, peroxidase, and 4- aminoantipyrine devoured the cholesterol to produce a colorless end product. In phase two, reagent 2 contains a second detergent that releases cholesterol from LDL-lipoproteins. This cholesterol forms a blue color complex when it combines with cholesterol esterase, cholesterol oxidase, and a chromogen system, which may be detected bichromatically at 540 and 660nm. The ensuring rise in absorbance is proportional to the sample's LDL-C content[24].

Determination of serum very low density lipoprotein-cholesterol (VLDL-C) concentration (mg/dL)

By dividing serum TG by five, the concentration of extremely low-density lipoprotein-cholesterol was estimated.(25)

VLDL- C concentration (mg/dL) = T G / 5.

Statistical analysis

Statistical analysis of data was performed using SAS (Statistical Analysis System - version 9.1). One-way ANOVA, two-way ANOVA [26]. And Least significant differences (LSD) post hoc test were performed to assess significant differences among means. $P < 0.05$ is considered statistically significant [27].

Results and Discussion

Total cholesterol (TC) mg/dl

The level of total cholesterol decreased significantly($P<0.05$) in the (G3)and (G4) groups during the 3rd and 4th month of experiment period compared with control group Cholesterol concentration decreased significantly($P<0.05$) in all trial groups with the progression of the trial period as shown in (Table 1).

Table (1): Effect of dietary (PSP) with or without SC on Total cholesterol mg/dl of local male lambs (M±SE).

months / animals/ group	zero time	1st month	2nd month	3rd month	4thmonth	5thmonth
G1 (control)	45.25±0.14 A a	44.31±0.29 A a	44.63±0.37 A a	41.62±0.2 8 B a	39.54±0.16 C a	35.46±0.14 D a
G2 (3%PSP)	45.16±0.17 A a	43.58±0.24 B a	43.97±0.48 B a	41.14±0.18 C ab	39.02±0.16 D ab	35.38±0.29 E a
G3 (3g SC+4%PSP)	44.86±0.27 A a	44.08±0.41 A a	44.12±0.23 A a	40.52±0.31 B b	38.44±0.07 C b	35.09±0.49 D a
G4 (3g SC+6%PSP)	44.93±0.27 A a	44.27±0.26 AB a	43.99±0.19 B a	40.61±0.35 C b	38.93±0.16 D b	35.42±0.11 E a
LSD`	0.78					

Means with a different small letter in the same column are significantly different ($P<0.05$)
Means with a different capital letter in the same row are significantly different ($P<0.05$)

Triglycerides (TG) mg/dl

The level of triglycerides recorded a significant ($P<0.05$) different among the experimental groups, as the concentration of triglycerides (TG) decreased in the 2nd, 3rd, and 4th treatment groups in the period of the third month and until the end of the experiment compared with control group as shown in (Table 2).

Table (2): Effect of dietary (PSP) with or without SC on Triglycerides mg/dl of local male lambs (M±SE).

month / animals/ group	zero time	1st month	2nd month	3rd month	4thmonth	5thmonth
G1 (con-trol)	22.55±0.4 A a	22.98±0.2 A a	22.56±0.1 A a	22.80±0.45 A a	23.32±0.22 A a	23.24±0.17 A a
G2(3%PSP)	21.02±0.1 A a	21.48±0.3 A a	21.34±0.3 A a	20.94±0.2 A b	21.32±0.4 A b	21.44±0.3 A b
G3 (3g SC+4%PSP	21.10±0.3 A a	21.92±0.2 A a	21.74±0.3 A a	21.62±0.3 A b	21.62±0.4 A b	21.56±0.3 A b
G4 (3g SC+6%PS)	21.08±0.21 A a	21.64±0.36 A a	21.38±0.39 A a	21.25±0.11 A b	21.02±0.38 A b	20.92±0.23 A b
LSD`	0.93					

Means with a different small letter in the same column are significantly different (P<0.05)
Means with a different capital letter in the same row are significantly different (P<0.05)

High density lipoprotein (HDL-C) mg/dl

As indicated in Table 3, the value of high-density lipoproteins increased significantly(P<0.05) in the G2, G3, and G4 treatment groups during the periods of the 2nd, 3rd, 4th and 5th months compared to control group while.HDL-c concentration decreased significantly(P<0.05) in all trial groups with the progression of the trial period.

Table (3): Effect of dietary (PSP) with or without SC on High density lipoprotein (HDL-C) mg/dl of local male lambs (M±SE)

months / animals/group	zero time	1st month	2nd month	3rd month	4th month	5th month
G1(control)	29.66±0.3 A a	28.46±0.54 B a	28.20±0.46 B b	25.24±0.05 C b	23.64±0.16 D b	19.64±0.13 E b
G2(3%PSP)	29.76±0.28 A a	29.08±0.07 A a	29.74±0.18 A a	27.10±0.12 B a	25.12±0.05 C a	21.88±0.22 D a
G3 (3g SC + 4% PSP)	29.68±0.46 A a	29.42±0.15 A a	29.90±0.56 A a	26.82±0.24 B a	25.06±0.04 C a	22.18±0.09 D a
G4 (3g SC+6%PSP)	29.70±0.34 A a	29.49±0.33 A a	29.76±0.33 A a	27.08±0.26 B a	25.68±0.10 C a	22.24±0.08 D a
LSD`	0.77					

Means with a different small letter in the same column are significantly different (P<0.05)
Means with a different capital letter in the same row are significantly different (P<0.05)

Low density lipoprotein (LDL-C) mg/dl

As indicated in Table 4, the value of low-density lipoproteins decreased significantly($P<0.05$) in the G2, G3, and G4 treatment groups compared to control group during the periods of the 2nd , to the 5th months. while, LDL-c concentration decreased significantly($P<0.05$) in treatment groups with the progression of the trial period.

Table (4): Effect of dietary (PSP) with or without SC on Low-density lipoprotein (LDL-C) mg/dl of local male lambs (M±SE).

months / animals/group	zero time	1st month	2nd month	3rd month	4th month	5th month
G1 (control)	11.27±0.25 A a	11.58±0.37 A a	11.93±0.62 A a	11.64±0.41 A a	11.24±0.07 A a	11.24±0.08 A a
G2(3%PSP)	11.09±0.22 A a	10.25±0.22 AB a	9.98±0.45 AB b	8.86±0.17 C b	9.62±0.11 BC b	9.22±0.05 BC b
G3 (3g SC+4%PSP)	10.99±0.22 A a	10.27±0.38 AB a	9.87±0.54 AB b	9.58±0.13 BC b	9.06±0.02 BC b	9.00±0.03 C b
G4 (3g SC+6%PSP)	11.14±0.30 A a	10.18±0.49 B a	9.93±0.17 B b	9.26±0.08 BC b	9.04±0.05 BC b	8.96±0.02 C b
LSD`	0.81					

Means with a different small letter in the same column are significantly different ($P<0.05$)
Means with a different capital letter in the same row are significantly different ($P<0.05$)

Very low-density lipoprotein (VLDL-C) mg/dl.

As indicated in Table 5, the value of very low-density lipoproteins decreased significantly($P<0.05$) in the 2nd, 3rd and 4th treatment groups, during the last three months of the experiment compared to its level in the control group.

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Table (5): Effect of dietary (PSP) with or without SC on Low-density lipoprotein (vLDL-C) mg/dl of local male lambs (M±SE)

months / animals/group	zero time	1st month	2nd month	3rd month	4th month	5th month
G1 (control)	4.51±0.09 A a	4.59±0.06 A a	4.52±0.03 A a	4.56±0.08 A a	4.66±0.04 A a	4.60±0.007 A a
G2(3%PSP)	4.20±0.03 A a	4.25±0.06 A a	4.26±0.07 A a	4.18±0.05 A b	4.26±0.09 A b	4.28±0.07 A b
G3 (3g SC +4 %PSP)	4.22±0.07 A a	4.37±0.05 A a	4.34±0.06 A a	4.33±0.06 A b	4.32±0.09 A b	4.31±0.07 A b
G4 (3g SC+6%PSP)	4.21±0.04 A a	4.32±0.07 A a	4.27±0.07 A a	4.25±0.06 A b	4.21±0.07 A b	4.22±0.06 A b
LSD`	0.20					

Means with a different small letter in the same column are significantly different ($P<0.05$)
Means with a different capital letter in the same row are significantly different ($P<0.05$)

The findings demonstrated that there were significant differences between the groups throughout the experiment in total cholesterol (TC), triglycerides(TG), LDL-C, and vLDL-C mean values. In contrast, there had been a significant increase in HDL-C values in the three treatment groups when compared to the control group, as show in tables [1,2,3,4,5].

Ruminants' digestion of fats is characterized by activities inside the rumen before they can be absorbed in the gut. The amount and makeup of fat exiting the rumen differs from that which was consumed due to the transformations that occur to fats while they are there, The decrease in cholesterol in all groups with the progression of the experimental period could be attributed to the effect of the shift in feeding lambs on green fodder [28], especially the alfalfa, as studies indicated the effect of a significant decrease in cholesterol with an increase in the period of feeding lambs on alfalfa, whether it was fresh or hay [29]. The reports attributed the cause of the effect of alfalfa to the saponins [30], as the saponins form an insoluble complex with cholesterol in the digestive system and increase its excretion in the faeces [31], Cholesterol and its level in the blood decrease with age [32] , and the reason for the decrease in cholesterol may also be attributed to the increased absorption of fats in the body of animals with age [33] and the high-efficiency lipoproteins in the blood decrease according to the decrease in cholesterol [34], and this conclusion agrees with [35, 36, 37].

The reason for the significant ($P<0.05$) decrease in cholesterol(TC), triglycerides(TG), low-density lipoproteins(LDL-c) and very low-density lipoproteins(vLDL-c) in treatment groups may be attributed to the antioxidant role of pomegranate seeds pomace , as it was found that the feed additives were rich in antioxidant compounds [38], and this conclusion is consistent with [39], which indicated that a decrease in cholesterol, triglycerides, low-density lipoproteins and very low-density proteins was observed after using pomegranate by- products , as it improves lipid metabolism and obstructs its oxidation.

Some scientific reports attribute the reason for reducing TC, TG, and LDL-c and vLDL-c to the fact that pomegranate seeds pomace(PSP) contain many active substances that affect lipid metabolism in the body [40]. PS contain an abundance of punicic acid, which plays an important role in lipid metabolism and lowers TC, TAG, LDL, and vLDL. by reducing it, the risk of atherosclerosis decreases as PSP improve the lipid profile [41]. This conclusion is consistent with both [42,43], which indicated the effect of punicic acid isolated from pomegranate seeds in reducing TC, TG, and LDL-c and vLDL-c in rats.

And some scientific reports mentioned the effect of PSP on lipid metabolism if they act as hypocholesterolemia, as adding them to the rabbit diet led to a reduction in TC and LDL-c and vLDL-c, and a significant increase in the concentration of HDL-c. The reason for this rise is explained by the effect of pomegranate seeds in activating the activity of parozonase 1, related to the production of HDL-c in the liv-

er, protecting it from harmful oxidation [38], or the reason may be due to the effect of the components of PSP of essential amino acids and fibers, which play an important role in addition to antioxidants in improving lipid profile, as they work together Fiber reduces TC and LDL-c in the blood, in addition to the effect of short-chain fatty acids available in PS, which play an important role in the synthesis of propionate acid, which reduces the formation of TC in the liver [44]. The amino acids in PSP are used as a source for the manufacture of HDL-c, which are contained in Its composition contains more than 50% of protein [45], in addition to the role of amino acids, which are lipotropic factors and are involved in the biosynthesis of protein, as well as in the synthesis of fatty acid β-oxidation enzymes in the body, antioxidant enzymes ,Superoxide dismutase (SOD) and catalase (CAT), and the rest of the protein bio factors, All of these enzymes activate the rate of lipid peroxidation in addition to their harmful antioxidant action, All these properties lead to the conclusion that the PSP protein has a hypolipidemic effect. by increasing the β-oxidation processes of fatty acids, thus lowering TC, LDL, and vLDL-c while raising HDL-c inside the body [46]. This conclusion is compatible with [47,48], which indicated the effect of pomegranate seeds in lowering TC, TG, LDL, and vLDL and increasing HDL-c in rats after adding pomegranate seed powder(PSP) by 10% to their diet.

Scientific reports [38] stated that the pomegranate fruit has a great ability to prevent and reduce the oxidation of HDL-c and increases their resistance to oxidation and maintenance, and this leads to an increase in their level in the blood, The phenols in PS also have a role in preventing lipid peroxidation through their direct effect by linking the phenols in pomegranate with LDL-c or indirectly by stabilizing the concentration of paraoxonase 1(PON1) enzyme in the blood serum related to HDL-c synthesis and paraoxonase (Pon2)enzyme in cells related to lowering TC concentration through the formation of compounds like ox-LDL, which is easily ingested by phagocytes, which ultimately leads to a decrease in TC and LDL-c in the blood [49]. Or, the reason may be due to the role of ellagic acid, one of the phenolics of PSP, which works to reduce the absorption of TC from the gastrointestinal tract and increase its excretion with faeces out of the body as well, or by blocking the action of 3 Hydroxy-3-Methyl-Glutaryl Co-A reductase (HMG-co-A) enzyme and sterol-0-acyltransferase, which has an important role in the mechanism of manufacturing TC and its metabolism in the body [50].

This result is supported by [7,8,9,47] which indicated a decrease in the level of TC, TG, LAD-c, and vLDL-c in rats after adding pomegranate by-products at 2.5% to their diets, as well as [52] noticed an improvement in the lipid profile where the TC, TAG, LDL-c, vLDL-c decreased significantly and the level of HDL-c increased in rats after being fed on a diet to which PSP were added by 10%.

Saccharomyces cerevisiae (SC) may act as an active agent in the rumen when pomegranate seed pomace (PSP) is added because PSP serves as a source of nutrients for SC, its significance may stem from its capacity to manipulate the rumen ecosys-

tem, particularly bacteria. For example, SC metabolic activity may feature particular bacterial strains that may inhibit bio-hydrogenation and reduce methane release, Additionally, it may be able to protect unsaturated fatty acids from oxidation in conjunction [52, 53]. Additionally, SC, which produces β-glucan in their cell wall, can lower TC levels in the blood plasma and liver to levels that are close to normal. The extract may lower blood plasma TG and TC levels. It is possible that the effect of β-glucans on fats is similar to the action of the fibers that work to disengage fats from the epithelial tissues in the small intestine, which leads to their excretion with the stool outside the body, thus reducing the absorption of TC and TG, accordingly decrease their concentration in the blood [54, 55, 56].

The reason for the decrease may be the effect of SC, which changes the fermentation of the rumen, as it increases the formation of short-chain fatty acids such as propionate, butyrate, and valerate, and this group of acids works to reduce the formation of TC and TG in the liver and may cause a change in the lipid profile and finally reduce the synthesis of TC and T G in the liver and blood [57]. or maybe the effect of SC, which increases the absorption of TC, T G, and LDL-c directly by the cells of the body [58].

Ooi and Liony, [59] who explained the way to remove TC by linking it with ions in the SC wall during its growth in the rumen, as cholesterol is then converted to coprostanol in the small intestine, so its absorption is reduced and it is excremented outside with the stool, and this leads to a decrease in the concentration of TC in the intestine and blood on the consecutive [60]. The concentrations of TC and TG decreased in calves after being raised on a ration to which SC was added [61],The results of our current study agree with those of [60,62 ,63,64,65], which indicated an improvement in the lipid profile in rams after feeding them on a diet supplemented with SC at an amount of 2 g / animal per day..

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