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EFFECT OF SUPPLEMENTING POMEGRANATE PEEL POWDER WITH CONCENTRATED DIET ON SOME METABOLITE MARKERS IN GOAT KIDS

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Abstract

This study aimed to evaluate the effect of pomegranate peel powder supplemented with a concentrated diet, at 0%, 2% and 4%, on body weight and several metabolite markers evaluated at three different times in goat kids. Eighteen weaned male of local goat kids aged approximately 3-4 months were used in this study. The goat kids were divided into three groups: control group with 0% pomegranate peel powder, treatment one supplemented with 2% pomegranate peel powder (20 mg/kg of dry matter), and treatment two supplemented with 4% pomegranate peel powder (40 mg/kg of dry matter). Blood samples were taken for analysis pre-treatment and at the end of each month during this study. A decrease in glucose level was seen after one month of adding 4% pomegranate peel powder to the diet. At the same time, insulin level declined in the third month of treatment with 4% pomegranate peel powder. Triiodothyronine and triiodothyronine/thyroxin ratio were reduced in the third month of treatment with 2% pomegranate peel powder, compared to control group. Although time had an impact on some variables, there were no further significant findings for the measurements of the lipid profile, liver enzymes, lipase, amylase, and total bilirubin. According to the results, treatment with 4% pomegranate peel powder lowered insulin and blood sugar levels in the first and third months. Triiodothyronine and triiodothyronine/ thyroxin levels were both decreased concurrently by supplementing 2% pomegranate peel powder without affecting thyroid stimulating hormone level.

Keywords: Blood Sugar, Goat Kids, Lipid Profile, Liver Enzymes, Metabolic Hormones, Pomegranates Peel Powders.

INTRODUCTION

Pomegranate (*Punica granatum L.*), a member of the *Punicaceae* family, have received a lot of attention as a source of nutraceuticals (Tzulker *et al.*, 2007). It is a small sized tree or a fruit-bearing deciduous shrub that is extensively grown and cultivated in many tropical and subtropical regions of the world (Morton, 1987). Pomegranate (PG) has long been utilized for therapeutic purposes and considered to have healing properties. It is included in the list of "functional foods", which play a vital role in reducing the risk of disease and slowing the progress of chronic diseases, in addition to their basic nutritional advantages. It is a rich source of dietary fiber, organic acids, vitamins, and minerals as well as phenolic components such flavonoids and tannins (Viuda-Martos *et al.*, 2010). The fruit contain valuable compounds in different parts. These parts can be divided into several anatomical origins: peel, seeds, and arils (Zhang *et al.*, 2010). Pomegranate peels (PGP), which include the husk and internal network membranes, make up about 50% of the fruit's weight (Caruso *et al.*, 2020). It is commonly considered as an agro-industrial waste with a potential source of valuable secondary plant

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metabolites and nutrients. It has stronger biological activity than the pulp, therefore, it is frequently utilized as animal feed. However, because the peel may contain moisture, most of it is wasted, contributing to an environmental problem (Magangana *et al.*, 2020). Pomegranate peels is an important source of bioactive compound such as water-soluble polyphenols, including mainly hydrolyzable tannins (e.g., punicalagins and punicalins) as well as phenolic acids, flavonoids, and pro- anthocyanidins (Safari *et al.*, 2018). Together, these compounds have potent antioxidant, antibacterial, antimutagenic, cardio-protective, apoptotic, and antigenotoxic properties, which may produce beneficial effects against a wide range of serious diseases (Viuda-Martos *et al.*, 2010) comprising diabetes mellitus and lowering the dyslipidemia caused by obesity and cardiovascular risk factors (Caruso *et al.*, 2020). Polyphenols are the main elements with anti-diabetic characteristics; these components lower blood glucose through a variety of mechanisms, including the reduction of glucose absorption through the intestine or peripheral tissues, although the most likely mechanism is the decrease of the enzyme glucosidase (Cheng and Liu, 2000). Pomegranate peel contains as much as three times the total amount of polyphenols, including condensed tannins and catechins, gallocatechins and prodelphinidins compared with the pulp (Ozcan *et al.*, 2011).

According to Safari *et al.* (2018), pomegranate seed pulp (peel + seed) supplementation led to a larger decrease in plasma glucose and triacylglycerol levels in transition dairy cows. Additionally, research by Viuda-Martos *et al.* (2010), showed that caffeic acid, another component of pomegranates, increases the uptake of glucose by rat adipocytes. Correspondingly, Faddladdeen and Ojaimi (2019), revealed that administration of pomegranate peel extract (PPE) had a protective effect on reducing blood glucose levels and protecting against hyperglycemia-induced hepatic changes. The possible effects of pomegranate peel on certain lipid profiles and hormones involved in metabolism in goats are not well understood. The purpose of the current study was to determine how adding different levels of pomegranate peel powder (0% as control, 2% PGPP as T1, 4% PGPP as T2) to a concentrated diet affect the lipid profile, liver enzymes, blood sugar levels and some metabolic hormones in growing goat kids.

MATERIALS AND METHODS

Location of the experiment

The experiment was carried out at the Animal Project, Department of Animal Production, College of Agricultural Engineering Science, and University of Duhok. The research protocol was approved by the research ethics committee at the Animal Production Department.

Experimental animals and diets

This study was conducted on eighteen weaned male of local goat kids aged approximately 3-4 months with body weight 14±0.3 kg were used in this study. Before starting the experiments, goat kids were quarantined for 12 days as an adaptation period and vaccinated with Polivac CL against enterotoxaemia. At the beginning of the experiment, all goat kids were vaccinated against external and internal parasites with Ivermectin 4% by subcutaneous injection. Levacloz

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was drenched against the external parasite and repeated 21 days later. After the adaptation period, goat kids were designated equality, and randomly into 3 treatment or groups (6 animals/group) for a period of 3 months. The first group was control, their diet containing no pomegranate peel powder (0% PGPP) as shown in Table 1. The second group (T1) received the same diet as control supplemented with 2% PGPP (20 g/kg of dry matter (DM)) and the third group (T2) supplied the same diet as control supplemented with 4% PGPP (40 g/kg DM). The goat kids were housed in individual cages (2 m^2) during the experimental period, and were weighed at weekly intervals. The diet consisted of two-part, concentrated diet (Table 1) which was weighed and fed to goat kids at 3% of their body weight twice a day at 9:00 and 17:00, a*d libitum* wheat straw was given at a rate of 300 g/ head/ day with free access to clean water. The diet formulated to meet the requirements of fattening goat kids according to (AFRC, 1993).

Diet chemical analysis

Chemical analysis for the concentrated diet, which contained barley, wheat bran, wheat, soybean, and pomegranate peel powdered, was used in this experiment as shown in table 1. The samples of the experimental diet that was offered and the refusals were collected and stored at room temperature for chemical analysis, as demonstrated in our previous work (Sulaiman *et al.*, 2022). Before chemical analysis, the obtained feed samples were grounded and oven-dried at 60°C for 48 hours. Chemical analysis performed for dry matter (DM), organic matter (OM), crude protein (CP), neutral detergent fibre (NDF), acid detergent fibre (ADF) and ether extract (EE) according to AOAC (Horwitz, 2000).

Table 1: The composition and chemical analysis of experimental diets

	Pomegranate peel powder (PGPP) in the diet levels (%)		
Ingredient (g/kg)	**Control 0% PGPP**	**T1 2% PGPP**	**T2 4% PGPP**
Barley	250	250	250
Wheat bran	350	350	350
Wheat	250	230	210
Soybean	150	150	150
Pomegranate peel powder (PGPP)	0	20	40
Chemical composition g/kg DM			
Dry matter	866	865	867
Organic matter	950	948	948
Crude protein	151	151	150
Ether extract	35	35	35
NDF	343	334	346
ADF	150	144	138
ME*	11.34	11.35	11.36

Control: (0% PGPP); **T1**: (2% PGPP or 20 g/Kg DM) of supplemented pomegranate peel powder; **T2**: (4% PGPP or 40 g/Kg DM) of supplemented pomegranate peel powder; **NDF**: neutral detergent fibre; **ADF**: acid detergent fibre; **ME**: metabolizable energy; *: predicted according to (AFRC, 1993).

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Preparation of pomegranate peel powder

Table 2, shows the chemical composition of pomegranate peels. The pomegranate peels were extracted, manually separated and air dried naturally. The dried peels crushed by grinder to powder. The peels powder was sieved and taken as raw materials. The dried PGPP was used with the concentrated diet in this study at a ratio of 2% (20 g/kg DM) for treatment one (T1) and 4% (40 g/kg DM) for treatment two (T2).

Table 2: Chemical composition of pomegranate peels (PGP)

Ingredient %	**PGP**
Organic matter	96.2
Crude protein	5.10
Ether extract	4.90
Total Ash	3.70
Crude fibre (CF)	11.2
Nitrogen free extract (NFE)	80.5
Metabolizable energy ME (MJ/kg)*	27.9

*ME was calculated according to (Mirzaei-Aghsaghali *et al.*, 2011).

Blood sample collection and analysis

The blood samples (5ml) were taken in the morning at 8:00 AM, before treatment (pre-treatment), and then once at the end of each month during the study period, following an overnight fast (12h).

The blood withdrawn from Jugular venipuncture for each goat kid by using disposable plastic vacutainers (Vaccutest, Italy). The samples were kept at 4˚C for 2 hours to clot, then they were centrifuged at 4000g for 15 minutes for serum separation and stored at -20˚C for further analysis. The serum was assayed using Auto Biochemistry Analyzer COBAS-6000 (Roche, Germany) for hormones (insulin; µIU/mL, TSH, T3 and T4; nmol/L), blood glucose (mg/dl), lipid profiles (total cholesterol, triglyceride, LDL and HDL; mg/dl), liver function test (ALT, AST, ALP; IU/L and total bilirubin; mg/dl) and the enzymes (lipase and amylase; IU/L) in Awny Lab. https://www.awnylab.com

Statistical analysis

Data were statistically analysed using Graph Pad prism 6 statistical software, expressed as means ± SEM. A two-way analysis of variance (ANOVA) followed by Tukey's post hoc test for multiple comparisons was used to assess the significance of variances of different levels of PGPP to concentrated diets on body weight, some metabolic hormones, lipid profiles, blood glucose levels, liver functions tests, lipase and amylase enzymes. P-value<0.05 was considered statistically significant.

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RESULTS

Body weight

There was no significant difference in body weight (B.W) of male goat kids between pomegranate treated groups (2% and 4%) with comparison to control (Figure 1). However, time had an evident significant increase effect ($P<0.0001$) on B.W in all group of animals from the first month to the end of the experimental period (third month).

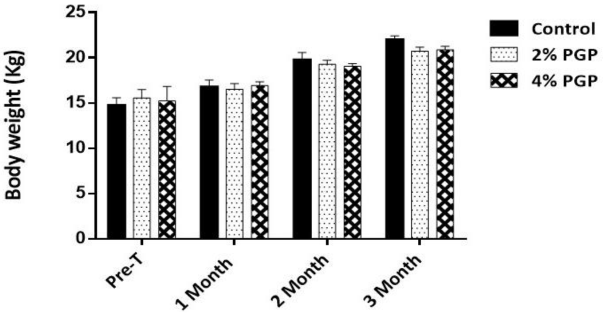

Figure 1: Changes in goat kids body weight (kg) in all studied groups throughout the experimental period. Each bar represents the mean ± SEM of six animals. Control= (no pomegranate peels powder supplementation, 0 % PGPP), treatment one (T1) with 2% PGPP (20 g /kg DM) and treatment two (T2) with 4% PGPP (40 g /kg DM)

Blood glucose and insulin levels

After one month of adding 4% PGPP to the diet, blood glucose levels were significantly lower ($P = 0.040$) compared to the control group, whereas insulin levels were significantly lower ($P = 0.05$) in the third month of treatment (Figure 2). A time effect was observed in both glucose ($P=0.0127$) and insulin ($p=0.05$) with group T2 presenting a lower level of glucose and insulin in month 1 and month 3, than control group, respectively.

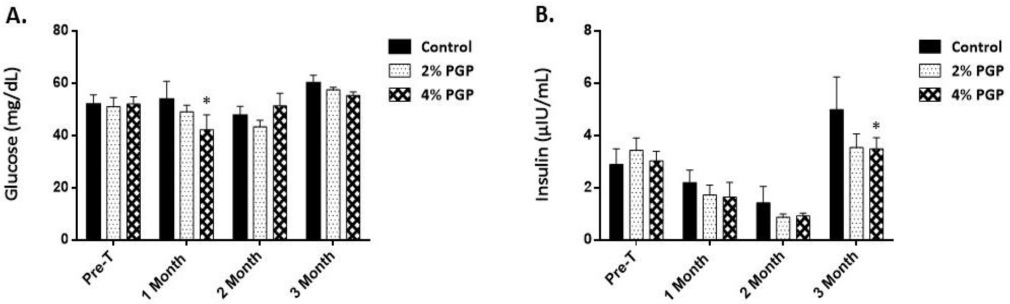

Figure 2: Effect of pomegranate peel powder supplement on (A) fasting blood glucose levels (mg/dl) and (B) insulin hormone (µIU/mL) in male goat kids. Each bar represents the mean± SEM of six animals. * Significant difference at P<0.05 level compared with the normal control group. Control= (0 % PGPP, no pomegranate peels powder supplementation), treatment one with 2% PGPP (20 g /kg DM), treatment two with 4% PGPP (40 g /kg DM)

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Lipase and amylase

The addition of 2% or 4% of PGPP to a concentrated diet had no impact on the lipase and amylase enzymes (Figure 3). Significant changes were taken into account as the study progressed (P=0.0052), (P=0.0348) for both lipase and amylase, respectively, as shown in figure 3.

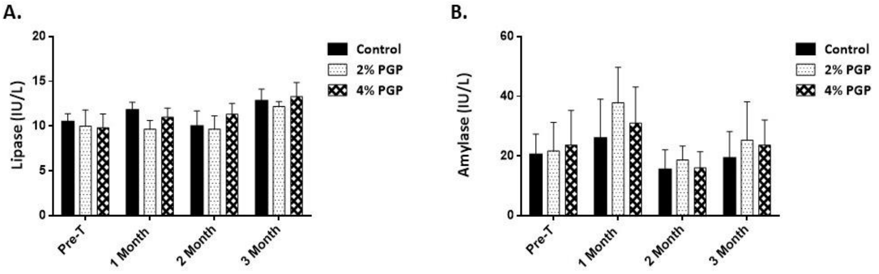

Figure 3: Effect of pomegranate peel powder supplement on (A) lipase and (B) amylase (IU/L) enzymes in male goat kids. Each bar represents the mean± SEM of six animals. Control= (no pomegranate peels powder supplementation, 0 % PGPP), treatment one with 2% PGPP (20 g/kg DM), treatment two with 4% PGPP (40 g/kg DM)

Lipid profile

Figure 4 shows that there were no substantial variances in the levels of triglycerides, cholesterol, HDL, and LDL between the treatment (2% PGPP and 4% PGPP) groups and the control group besides that the duration of the experiment had no impact on the lipid profiles either (Figure 4).

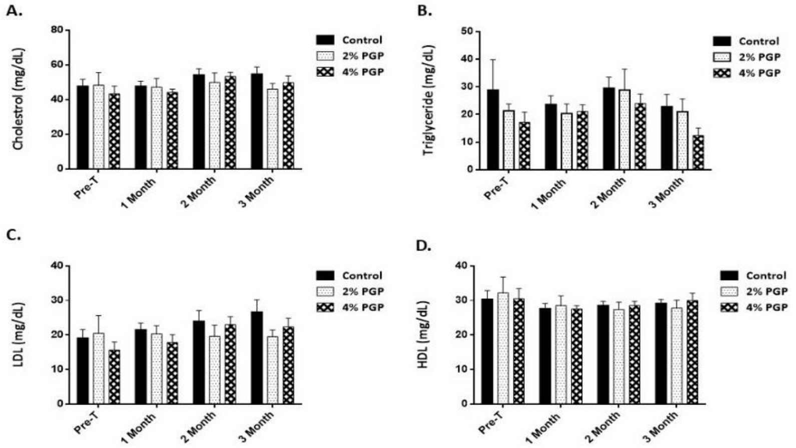

Figure 4: Effect of pomegranate peel powder supplement on lipid profile; (A)Triglyceride, (B) cholesterol, (C) HDL and (D) LDL (mg/dl) in male goat kids. Each bar represents the mean ± SEM of six animals. Control= (no pomegranate peels powder supplementation, 0 % PGPP), treatment one with 2% PGPP (20 g/kg DM), treatment two with 4% PGPP (40 g/kg DM)

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Thyroid hormone

Triiodothyronine (T3) and the T3/T4 ratio in the current study treated with 2% PGPP significantly decreased by the third month of this experiment compared to the control group (P<0.0001 and P=0.006), respectively (Figure 5). While 4% PGPP treatment for T3 and T3/T4 in the same month shown a significant increase (P=0.022) in comparison to 2% PGPP treatment (P=0.038). As the experiment progressed, there was a significant effect of time on T3 (P<0.0001), as seen in figure 5. The level of thyroxin (T4) was significantly lower in the pre-treatment (4% PGPP) group compared to control, despite the fact that there were no alterations between the treatment groups (2% and 4%) PGPP as the experimental progressed. There were no differences in TSH levels between the treatment groups (2% and 4%) PGPP and the control.

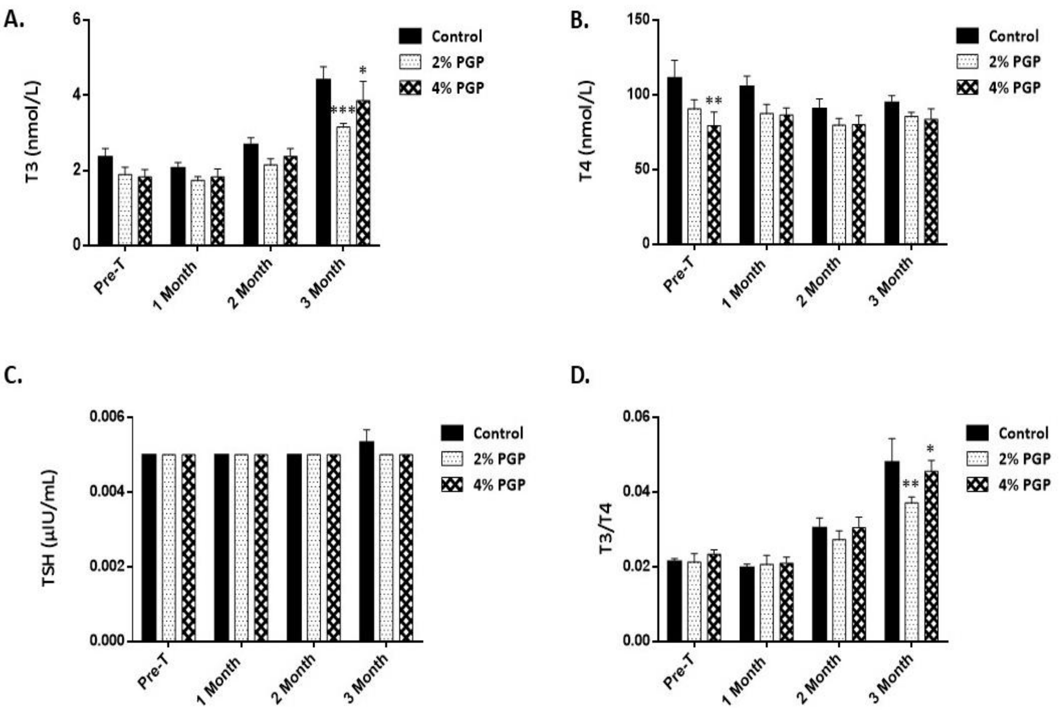

Figure 5: Effect of pomegranate peel powder supplement on thyroid hormone; (A) Triiodothyronine (T3, nmol/L) and (B) Thyroxin (T4, nmol/L), (C) T3/T4 ratio and (D) TSH level (µIU/mL) in male goat kids. Each bar represents the mean± SEM of six animals. * Significant difference at P<0.05 level compared with the normal control group. ** P<0.01 and **** P<0.0001. Control= no pomegranate peels powder supplementation (0% PGPP), treatment one with 2% PGPP (20 g/kg DM), treatment two with 4% PGPP (40 g/kg DM)

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Liver enzymes and total bilirubin

According to the current investigation, neither the 2% PGPP nor the 4% PGPP treatments significantly changed the levels of the AST, ALT, ALP, or total bilirubin compared to the control group (Figure 6). However, there was a noticeable decline in pre-treatment AST values between treatment groups and controls (P=0.02). A significant (P=0.0035) decrease in AST was also seen over time as the experimental progressed (Figure 6).

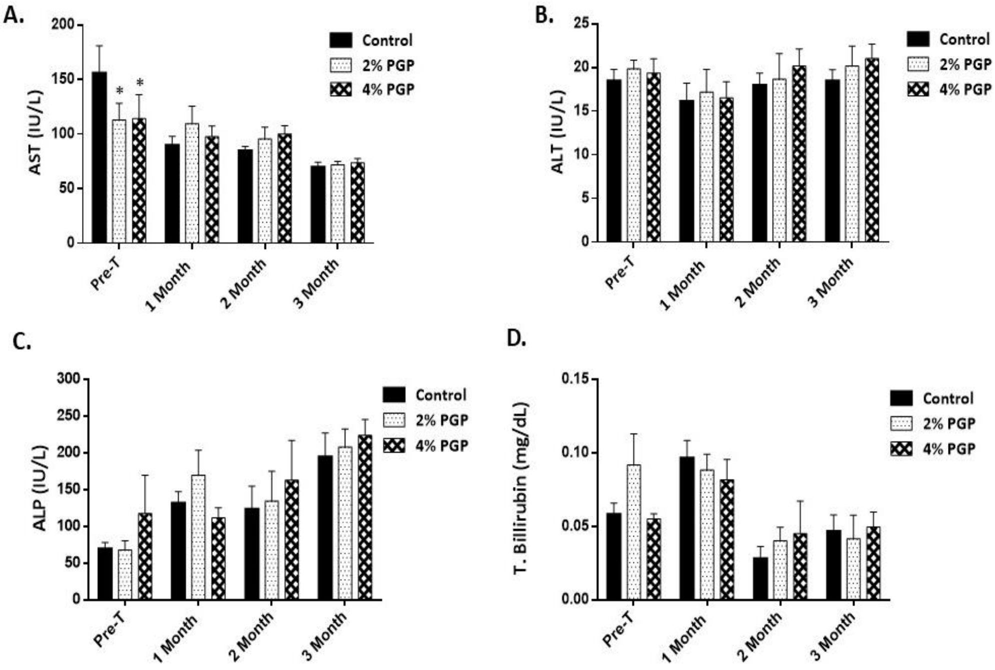

Figure 6: Effect of pomegranate peel powder supplement on liver enzymes; (A) Aspartate transaminase (AST) concentration (IU/L), (B) Alanine transaminase (ALT) concentration (IU/L), (C) Alkaline phosphatase (ALP) concentration (IU/L), and (D) Total bilirubin (mg/dl) in male goat kids. Each bar represents the mean ± SEM of six animals. * Significant difference at P<0.05 level compared with the normal control group. Control= (no pomegranate peels powder supplementation, 0 % PGPP), treatment one with 2% PGPP (20 g /kg DM), treatment two with 4% PGPP (40 g /kg DM)

DISCUSSION

Effects on body weight

Body weight of goat kids was not changed with supplementing their diet with 2% or 4% PGPP. In agreement with the obtainable results Saeed *et al.* (2018), observed no significant effect of different level of PGP (0%, 1.5% and 3%) on weight gain in Awassi lambs. The final body

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weight of lambs fed diets supplemented with PGP at the rates (2%, 4%, and 6% PGP) did not change, according to a study by Hussein and Shujaa (2013).

In contrast to the current results, Omer *et al.* (2019), revealed that adding dried pomegranate peel to complete feed mixtures at various doses (0.5, 1.0, and 2.0%) increased body weight in growing Ossimi lambs. These findings conflict with those of Sadq *et al.* (2016), who revealed that final B.W of Karadi lambs fed 1% or 2% pomegranate peel was significantly higher than that of lambs fed 4%. Tannins typically cause a decrease in palatability, which discourages grazing (Ngwa *et al.*, 2003). Cattle, sheep, and goats are less likely to favor some plants due to high tannin levels (Perevolotsky *et al.*, 1993). While Amri *et al.* (2020), attributed the reduction in body weight in animals treated with PGP to the decrease in intestinal carbohydrates absorption, which contributes to the decrease in energy intake leading to the weight loss.

Effects on glucose and insulin levels

Pomegranate has been shown to have hypoglycemic effects in both *in vitro* and *in vivo* studies, including improved blood lipid profiles, lower total cholesterol and increase insulin sensitivity. It also affects the activity of glucose transporter type 4 and inhibits α-glucosidase (Medjakovic and Jungbauer, 2013). The majority of pomegranate products have a hypoglycemic effect, according to earlier research done on both humans and experimental animals (Sohrab *et al.*, 2019).

In the present study, glucose and insulin levels decreased after one and three months of treatment with 4% PGPP. Our results are consistent with previous studies that validated the PGP significantly reduced blood glucose levels and had a protective effect (Saad *et al.*, 2015; Mphahlele *et al.*, 2016). Pomegranate by-product feeding lowered postpartum plasma glucose levels in cows, as reported by Safari *et al.*(2018), which may be related to antioxidant status or insulin sensitivity. As a result, there is a possibility that animals fed pomegranate seed pulp (PSP) had improved insulin sensitivity, which led to more glucose uptake and less fat mobilization (Safari *et al.*, 2018).

However, Hussein and Shujaa (2013), showed that the various dosages of PGP (2, 4 and 6%) had no effect on blood glucose levels in Awassi lambs. Furthermore, there were no appreciable differences in serum glucose levels between the goats given 1% PGP and the control group, according to Kholif *et al.* (2022). Pomegranate peels may have an inhibitory effect on intestinal glucose absorption, which would explain why it has a hypoglycemic effect (Saad *et al.*, 2015). According to Chau *et al.* (2003), peels contain soluble dietary fiber, which may have the same glucose-lowering effects as viscous fiber. While Amri *et al.* (2017), ascribed the anti-diabetic effects of PG components to phenolic compounds, tannic acids, gallic acid, ellagic acids, and flavonoids that have hypoglycemic properties. The anti-hyperglycemic effects of PG may be achieved through stimulating insulin secretion or by enhancing insulin action.

Pomegranate peel may also have a further pancreatic mechanism of action that enhances pancreatic β-cell activity and, as a result, increases insulin secretion (Papaccio *et al.*, 2000). The ability of PPE to lower blood sugar levels has been hypothesized to result from improved insulin receptor sensitivity, increased peripheral glucose uptake, and inhibition of the proximal

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tubular reabsorption mechanism for glucose, rather than from the potential for insulin release from pancreatic cells (Belkacem *et al.*, 2010). Pomegranate peel treatment also restored and regenerated pancreatic cells and revealed normal-sized islets of Langerhans (Saad *et al.*, 2015). The improvement in insulin sensitivity observed in pomegranate-treated animals may be due to the anti-inflammatory properties of the fruit extracts (Amri *et al.*, 2020).

Effects on lipase and amylase

In the current study, pomegranate peel powder supplemented to a concentrated diet at the levels 2% and 4% had no effect on lipase and amylase. In contrast to our findings, Amri *et al.* (2020), reported that rats receiving long-term PGP HFD therapy had markedly lower levels of amylase and lipase activity than rats in the HFD group. Pomegranate extracts have been reported to suppress amylase activity (Amri *et al.*, 2020), possibly due to the presence of active compounds that are assumed to be pancreatic lipase inhibitors (Les *et al.*, 2018). Pomegranate extract supplementation may have improved plasma lipid profiles because lipase, a vital enzyme in lipid metabolism, is inhibited, which reduces the absorption of fat and cholesterol (Amri *et al.*, 2020).

Effects on lipid profiles

Lipid profiles were unaffected by the 2% and 4% PGPP treatment in this experiment. These results were consistent with those reported by Khan *et al.* (2015), who noticed that Karadi lambs fed PGP at different levels (1, 2, and 4%) did not considerably differ from the control group in terms of total cholesterol, HDL, or triglyceride concentration. Similar to our results, Hussein and Shujaa (2013), revealed no difference in cholesterol and triglyceride levels between PGP concentrations of 2, 4, and 6% in Awassi lambs. Consequently, Kholif *et al.* (2022), indicated that lactating goats fed 1% PGP had no significant variations in blood cholesterol, triglycerides, HDL, LDL, or the LDL/HDL ratio. It has been reported that pomegranate extract with the right active components regulates lipid metabolism dysregulation and eventually relieves associated disorders (Yang *et al.*, 2018).

Contrary to the results of the present study, other studies (Saad *et al.*, 2015; Shujaat and Hussain, 2016) found that serum levels of cholesterol, triglycerides, and LDL were significantly lower, whereas serum HDL levels were higher, in the PGP group of animals compared to the control group. Pomegranate peel extract was shown to have a lipid-lowering effect after being administered to diabetic rats; total cholesterol and triglyceride levels significantly decreased (Belkacem *et al.*, 2010).

This variation in lipid profile levels could be caused by a number of factors, including the genotype, age, and physiological condition of the experimental animals, modifications to the PGP doses used in these studies, changes to the treatment time, and diverse treatment techniques.

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Effects on thyroid hormone

The results of the present study agreed with those reported by Gonçalves *et al.* (2013), who established that short-term treatment with flavonoid (rutin) *in vivo* was able to reduce serum thyroid hormone (T3 and T4) concentration in rats without affecting serum TSH levels.

Conflicting to our results, Ramzi (2016), observed no significant effects of different levels of pomegranate peel (0, 1, 2 or 4%) in the diet of Karadi lambs on T3, T4 and TSH. On the other hand, Al-Kattan *et al.* (2018), detected that pomegranate treatment enhanced T3 levels in rabbits, which contrast with our results. The increasing effect of the high dose of ellagic acid extracted from PG on thyroid hormone (T3 and T4) secretion was confirmed by Arrak (2010). This effect may be attributable to ellagic acid's antioxidant activity, which decreases or prevents the adverse effects of the hypothalamic-pituitary-thyroid axis or thyroid gland directly. It could be because pomegranates contain flavonoids like rutin (Al-Kattan *et al.*, 2018), which may increase the TSH hormone's gene expression and pituitary gland output (Gonçalves *et al.*, 2013). Or maybe because pomegranates have vitamins E and C, which cause the pituitary gland to release the hormone TSH, which in turn causes the thyroid gland to release T4 and T3 (Oboh *et al.*, 2007).

Effects on liver enzymes and total bilirubin

Aspartate aminotransferase (AST) is not a hepatocyte specific enzyme, it is also produced in other organs. Alanine aminotransferase (ALT), which is a specific enzyme for liver cells released by hepatocellular damage, is used to assess liver dysfunction (Al-Shaaibi *et al.*, 2016). Alkaline phosphatase (ALP) is used to detect bile obstruction, i.e. mild and progressive damage to the liver. However, ALT and ALP are considered to be more specific than AST in monitoring liver functions (Ramzi, 2016).

The results this study were similar with those obtained by Ramzi (2016), who found that PGP at various doses (0, 1, 2, or 4%) had no substantial effect on the levels of AST, ALT, or ALP in Karadi male lambs. Neither the AST nor ALT levels of lactating goats fed PGP in the ration with or without polyethylene glycol changed significantly, according to Kholif *et al.* (2022). On the other hand, other studies (Saad *et al.*, 2015; Ramzy, 2019) detected a significant reduction in serum levels of both AST and ALT enzymes in animal given pomegranate peel. This is can be explained by the hepato-protective effect of pomegranate peel. This effect is might be due to antioxidant content of pomegranate juice and peel extract. The presence of polyphenolic compound in pomegranate was proved to play a role in the protective activity that seemed to be linked to its effective antioxidant activity (Bassiri-Jahromi, 2018).

CONCLUSION

This study demonstrates that supplementing a concentrated diet with 4% PGPP decreased blood glucose and insulin levels in male goat kids at the first and the third months of this experiment. While 2% PGPP reduced T3 and T3/T4 ratio without affecting TSH levels. The other variables, did not show any appreciable alterations.

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Conflict of interests

The author declares that there is no conflict of interest.

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