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The Influences of Adding Polyethylene Glycol and Activated Sodium Bentonite on the Performance, Blood Parameters, and Muscle Mineral Content of Saanen Goats Fed Pistachio Byproducts

Research Article

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ABSTRACT

This study was conducted to evaluate the impact of dietary addition of polyethylene glycol (PEG) or activated sodium bentonite as tannins deactivation materials on the performance of Saanen goats fed diets containing pistachio by-products (PBP). Twenty-one Saanen male goats (27±3 kg, 10 months) were assigned to three dietary treatments in a completely randomized design and fed for 60 days. Three experimental diets consisted of a diet containing 30% dry matter (DM) pistachio by-products with no additive (control); control diet supplemented with PEG at 1.0% of DM (PEG group), and control diet supplemented with activated sodium bentonite at 1.0% of DM (G-bind group). Results indicated that dry matter intake (DMI), total gain, average daily gain (ADG), and ruminal pH were not affected by treatments ($P\geq0.05$). There were no significant differences among treatments in hematological parameters ($P\geq0.05$) except monocyte count ($P<0.05$). The plasma concentrations of total triglycerides (TG) decreased ($P<0.05$) by adding G-bind. Serum Insulin concentration was also increased significantly ($P<0.05$) in the PEG group compared to the control. Besides, the G-bind increased the calcium content of this muscle compared to the control and PEG group ($P<0.05$). Zinc content in muscle showed a significant increase in the PEG treatments in comparison to other experimental groups ($P<0.05$). Furthermore, Iron (Fe) content in muscle improved by both additives ($P<0.05$). Data showed that there were no significant differences among treatments for hair fiber characteristics ($P\geq0.05$). It can be concluded that activated sodium bentonite can be an appropriate substitute for PEG as a tannin-deactivation material in diets containing 30% DM PBP for feeding goats.

KEY WORDS pistachio skin, polyethylene glycol, polyphenolic, Saanen goat, sodium bentonite, tannin.

INTRODUCTION

Livestock breeding and nutrition in Iran as a country with an arid and semi-arid climate has special difficulties in terms of providing fodder for livestock feed. Livestock that are breeding in warm and semi-arid regions are subject to severe fluctuations in the quantity and quality of feed throughout the year (Sejian *et al.* 2012). Therefore, the use of agricultural by-products as animal feed has recently been considered as a solution to this problem. So, pistachio by-products can be considered as an important source of feed for ruminant nutrition, especially when there are many restrictions on providing feed requirement of livestock animals due to green forages scarcity during the dry periods and cold winter. Pistachio by-products are composed of soft external hulls, twinges, leaves, and some kernel and bony shells remaining from the de-hulling process of the crop (1.25-2 kg PBP/kg dry pistachio) (Forough Ameri and

Shakeri, 2008). The annually fresh PBP obtained in Iran is estimated at approximately 500000 tonnes (Shakeri *et al*. 2013). However, due to having a high content of tannins and polyphenolic compounds in PBP (Seied Moemen, 2003), and problems related to its drying and maintenance, the use of this by-product by ruminants might be restricted (Forough Ameri and Shakeri, 2008).

The total phenolic compounds (7.6-15.6%) and total tannins (3.4-10.15%) of sun-dried pistachio by-product have been reported by different researchers (Bagheripour *et al*. 2008; Ghaffari *et al*. 2014; Sedighi-Vesagh *et al*. 2015). Pistachio by-products used in the present study had relatively higher levels of total tannins (6.44% of DM) and phenolic compounds (10.37% as DM basis) as anti-nutrients that may reduce the ruminal degradation of protein and energy availability (McNabb *et al*. 1996). These anti-nutrients in long-term feeding may have adverse effects on the health and growth performance of animals. For example, it has been reported that consumption of some feedstuffs with high levels of hydrolyzable tannins has the potential to cause gastrointestinal hemorrhage, hepatic necrosis, and kidney failure in animals (Mahgoub *et al*. 2008a). The level of tannin consumption plays a decisive role in the occurrence of these damages. Moreover, as the amounts of tannins increases in forage species, their palatability and digestibility decrease, and consequently feed intake from these tannin containing forages is reduced (Silanikove *et al*. 1996a). Some studies that investigated the effect of PBP in replacement of alfalfa hay on Saanen goats performance, showed that the goats fed PBP had a lower DMI (Ghaffari *et al*. 2013) and crude protein (CP) digestibility than other treatments (Ghaffari *et al*. 2013; Sedighi-Vesagh *et al*. 2015). Tannin-binding agents such as polyethylene glycol (PEG) act as a substance that can limit tannin bioavailability and thereby improve nutrient digestibility. Polyethylene glycol as an inert unabsorbed polymer can bind to tannins over a wide range of pH and prevents the formation of tannin-protein complexes and can even displace protein from a pre-formed tannin-protein complex (Frutos *et al*. 2004). Therefore, PEG has been used to alleviate the negative effects of condensed tannins (CT) in sheep, cattle, and goats kept at maintenance level. However, PEG is very expensive, especially in developing countries (Ghandour *et al*. 2014); besides it may reduce the positive effects of tannins on reducing methane production and internal parasites (Bhatta *et al*. 2009). So, the use of substances as a substitute for polyethylene glycol in the tanniniferous diet of ruminants can be considered. The physical and chemical structures of smectite clays let them absorb mycotoxins, bacteria, viruses, ammonia, pesticides, heavy metals, phenol, and tannins and dismiss them from the body. Bentonite is a common smectite clay mineral fed to ruminants for this purpose (Huwig *et al*. 2001; Nadziakiewicza *et al*. 2019). Therefore, sodium bentonite may be appropriate as an alternative to PEG, for its phenol and tannins adsorption ability. In a study by Anirudhan and Ramachandran (2006) on the adsorption of tannins by cationic bentonite, they stated that cationic bentonite could be an effective surface adsorbent for the purification of water contaminated with tannins. Also, Ghandour *et al*. (2014) demonstrated that using bentonite as a tannins deactivation material may have some capability similar PEG, especially with the low price of bentonite and its activity to adsorb other harmful substances that may be occurred in feedstuffs. Moreover, it has been reported that sodium bentonite can decrease ruminal ammonia concentration, and improve feed and bacterial protein flow to the small intestine (Ivan *et al*. 1992b). However, limited information is available on the tannins deactivation properties of sodium bentonite as a feed additive in ruminant nutrition.

The present study aimed to investigate the influences of dietary supplementation of PEG or activated sodium bentonite (G-bind) as deactivation of tannins in the diet containing pistachio by-products on performance, blood metabolites, muscle mineral composition, and fiber characteristics of Saanen goats.

MATERIALS AND METHODS

Twenty-one clinically healthy young male Saanen goats aged about 10 months, with an initial weight of 27 ± 3 kg, were allocated to 3 dietary treatments in a completely randomized design by the stratified randomization based on their live body weights. The experimental period lasted 60 days (15 d for adaptation, 45 d for measurements of live weight and feed intake) from 20 January to 20th of March. This experiment was performed in the animal and poultry research center of Ferdowsi University of Mashhad (Mashhad, Iran). Animals were kept in individual pens in a barn, protected from rain and wind, and equipped with individual troughs to facilitate quantitative measurement of feed intake. Goats were cared in accordance with guidelines of the Iranian Council of Animal Care (1995). Before initiating the experiment, goats were treated according to manufacturer recommendations with an anthelmintic (Valbazen; Smith, Kline and Beecham, Philadelphia, PA) and a coccidiostat (Amprolin; Aguet, Rahway, NJ) (Walz *et al*. 1998). The dietary treatments were complete diets from the feedstuffs indicated in Table 1. Three experimental diets were: (1) diet contains 30% DM pistachio-by products with no additive (control); (2) control diet supplemented with PEG at 1.0% of DM (PEG group); (3) control diet supplemented with activated sodium bentonite at 1.0% of DM (G-bind group). Activated sodium bentonite with the brand

name of G-bind that was used in this experiment is provided by Paya Farayand Hezare Novin Company in Mashhad, Khorasan Razavi, Iran. Diets were adjusted using (SRNS 1.9) software. Total Mixed Rations (TMR) with 50:50 forage (20% corn silage and 30% pistachio-by products) to concentrate ratio were prepared and formulated to have similar metabolizable energy (ME), ether extract (EE), CP, neutral detergent fiber (NDF), and non-fiber carbohydrates (NFC) (Table 1). The PBP and dietary samples were analyzed for DM, EE, and CP (AOAC, 2002), NDF and acid detergent fiber (ADF) (Clovis *et al.* 2008), NFC (NRC, 2001), total phenols (TP), and total tannins (TT) (Makkar, 2003). The chemical composition of pistachio-by products is shown in Table 2. The fresh pistachio by-products were collected (in September) from pistachio de-hulling factories in Feizabad (Khorasan Razavi Province, Iran) which is located on the northeast part of Iran at 351010N latitude and 581780E longitude. Other ingredients that are used in the diets were provided from local markets at Mashhad (Khorasan Razavi, Iran.)

Goats were individually housed in individual pens and were allowed *ad libitum* access to feed and water. Feed intakes and feed refusals were collected before the morning feeding and weighed daily during the measurement period. Experimental animals were also weighed at the beginning and end of the experiment to examine weight gain. For this purpose 14 to 16 hours before weighing, goats were deprived of feed and the average daily weight gain was calculated. Rumen fluid samples were taken from animals by stomach tube with a vacuum pump 4 h after the morning feeding on the last day of the experiment and were checked to have no saliva. The pH was measured immediately with a portable digital pH meter (METROHM 691).

On the last day of the experiment, two sets of blood samples (5 mL) were taken from each animal via jugular venipuncture using a 5 mL syringe. A 5 mL blood sample was collected into labeled sterile bottles containing EDTA as an anticoagulant for the determination of hematological parameters. Blood samples for serum analysis were collected into anticoagulant-free bottles, allowed to coagulate at room temperature, and centrifuged at 1500 × g for 10 minutes (SIGMA 2-16 PK). The supernatant sera were then harvested and stored in a freezer for subsequent biochemical analysis. Plasma metabolites were determined using commercial kits (Pars Azmoon Company, Tehran, Iran) and an auto-analyzer (BIOSYSTEMS A15, Poland).

At the end of the experimental period (60 days), longissimus dorsi muscle biopsy samples were obtained from the 12th–13th rib interface following the procedure explained by Malau-Aduli *et al*. (1998).

Briefly, the animal was steered into a weighing chute with collapsible sides and some head restraint.

The longissimus dorsi muscle area on the back of the animal between the 12th and 13th ribs was shaved with a small electric clipper and washed with 90% ethanol and chlorhexidine. About 15 mL of a local anesthetic agent, lignocaine was administered intramuscularly. Five minutes following the administration of the anesthetic, a 5-7 cm incision was made with a scalpel blade and about 5 g of the underlying fat and *Longissimus dorsi* muscle was sampled. The wound was closed via 3-4 interrupted sutures using surgical thread. An anti-bacterial aerosol, Cetrigen, was used to the sutured area on the skin to promote wound healing, prevent flies and the animal was released back to the stockyard. No postoperative complications were reported as healing was rapid. The sutures were removed after 10-14 days. The muscle biopsy sample was immediately stored in a plastic bag on dry ice, flushed with nitrogen gas, and assigned into a portable icebox. Samples were homogenized and frozen at 20 ˚C until the analyses were performed. The muscle biopsies were analyzed for mineral composition. The Zn, Cu, Ca, and Fe contents were determined on *Longissimus dorsi* from the loin region using a flame atomic absorption spectrophotometer (Varian Specter AA 50B, Varian Ltd, Pty, Australia) (Jorhem, 2000).

For measuring the properties of the hair fibers (length and diameter of hair fibers and their washing efficiency) in goats, patches of fiber from defined areas (10 cm×10 cm) were repeatedly shorn on the first and last day of the experiment from the right mid side of each animal. To measure the length of the fibers, the length of 30 single fibers was determined by placing them on a calibrated glass plate so that there was no curl. The micro projection reference method was used to measure the fiber diameter (American Society for Testing and Materials, 1978). The percentage of clean fiber weight was estimated according to Khan *et al*. (2012).

Briefly, 10 g of the hair sample from each goat was taken and after removing its impurities, samples were dried in an oven (105 ˚C for 60 min). They were then washed after being placed inside the bag. After washing (in water containing sodium carbonate and non-ionic detergent), wool samples were dried for 16 h at 70 ˚C and any visible exogenic particles (vegetative matter) were removed using clean polypropylene forceps. Washing efficiency was calculated from the ratio of the weight of the washed fibers to the initial weight.

Statistical analyses

For this experiments data were analyzed by SAS (2003) using the GLM procedure as a completely randomized design with 3 treatments and 7 replications. Significant differences between individual means were identified using Duncan's multiple range test at a 5% probability level.

Table 1 Feed ingredients and chemical composition of experimental treatments (%DM)

Ingredients	Treatments		
	Control	PEG	G-bind
Corn silage	20	20	20
Pistachio by-products	30	30	30
Barley grain	25	27	27
Canola meal	15.5	16	16
Wheat bran	8	4.5	4.5
Calcium carbonate	0.5	0.5	0.5
Vitamin-mineral supplement	0.5	0.5	0.5
Salt	0.5	0.5	0.5
Poly ethylene glycol (PEG)	0	1	0
Activated sodium bentonite (G-bind)	0	0	1
Sum	100	100	100
Chemical composition (%DM)			
Dry matter	96.87	97.03	97.21
Metabolizable energy (Mcal/kg DM)	2.68	2.53	2.53
Crude protein	14.40	14.30	14.30
Ether extract	4.20	4.10	4.10
Non-fiber carbohydrates (NFC)	41.20	41.30	41.30
Neutral detergent fiber (NDF)	35.50	34.20	34.20
Acid detergent fiber (ADF)	21.00	20.70	20.70
Calcium	0.6	0.6	0.6
Phosphorus	0.5	0.5	0.5

Table 2 Chemical composition of pistachio by-products

Composition	Percentage of dry matter
Dry matter (%)	94.21
Crude protein	12.28
Ether extract	6.73
Neutral detergent fiber (NDF)	36.45
Acid detergent fiber (ADF)	26.32
Ash	11.89
Total phenolic compounds	10.37
Total tannins	6.44
Condensed tannins	1.27

RESULTS AND DISCUSSION

Feed intake, average daily gain (ADG), and rumen pH are presented in Table 3. Dry matter intake (DMI), total gain, and ADG were not affected by treatments ($P \geq 0.05$). The ruminal fluid pH was also similar among treatments ($P \geq 0.05$).

The mean of hematological parameters is presented in Table 4. There were no significant differences among treatments mean in hematological parameters except monocyte count. The plasma concentrations of total TG decreased ($P<0.05$) by adding G-bind to a diet containing 30% PBP (Table 5). Serum Insulin concentration was also increased significantly ($P<0.05$) in the PEG group compared to the control.

Other biochemical and hormonal measurements were not significantly different among the experimental group (Table 5).

The mineral composition of the longissimus dorsi muscle samples from goats carcasses are shown in Table 6. There were significant differences in the mineral composition of muscle among the treatment groups ($P<0.05$). The G-bind increased the calcium content of this muscle compared to the control and PEG group ($P<0.05$). Zinc content in muscle showed a significant increase in the group containing PEG in comparison to other experimental groups ($P<0.05$). Iron content in muscle also differed among the experimental group and improved by both supplementations of PEG and G-bind to the basal diet containing PBP as a source of fodder ($P<0.05$).

Table 3 Dry matter intake, rumen pH, weight changes and average daily gain of goats fed different treatments

Chemical composition (%DM)	Treatments			SEM	P-value
	Control	PEG	G-bind		
Dry matter intake (DMI) (g/d)	407.60	347.12	401.26	45.012	0.601
Rumen pH	6.17	6.15	6.23	0.135	0.918
Initial weight (kg)	27.20	27.00	27.15	1.940	0.996
Final weight (kg)	32.21	29.34	32.10	2.030	0.571
Total gain (kg)	5.01	4.43	4.90	0.513	0.783
Average daily gain (ADG) (g/d)	166.80	141.50	163.20	16.564	0.572

PEG: polyethylene glycol and G-bind: activated sodium bentonite.
SEM: standard error of the means.

Table 4 Effect of different treatments on hematological parameters in Saanen goats

Parameters	Treatments			SEM	P-value
	Control	PEG	G-bind		
WBC (10^2/µL)	154.00	130.00	111.00	16.359	0.238
Hb (g/dL)	9.92	10.42	10.04	0.242	0.346
PCV (%)	34.80	36.60	35.01	0.931	0.357
MCHC (g/dL)	30.51	30.50	30.73	0.612	0.956
Neutrophil (μL^{-1})	26.20	28.80	26.60	5.380	0.934
Lymphocyte (per/µL)	7300.00	6900.40	7100.80	500.307	0.887
Monocyte (per/µL)	2.50^{b}	6.00^{a}	5.00^{a}	0.507	0.021
Fibrinogen (mg/dL)	360.00	312.00	360.00	35.137	0.553

PEG: polyethylene glycol; G-bind: activated sodium bentonite; WBC: white blood cell; Hb: hemoglobin; PCV: packed cell volume and MCHC: mean corpuscular hemoglobin concentration.
The means within the same row with at least one common letter, do not have significant difference (P>0.05).
SEM: standard error of the means.

Table 5 Effect of different treatments on plasma metabolites, liver enzymes, and hormones in Saanen goats

Parameters	Treatments			SEM	P-value
	Control	PEG	G-bind		
Plasma metabolites					
Glucose (mg/dL)	57.71	52.15	53.85	2.005	0.303
Blood urea nitrogen (mg/dL)	23.61	21.58	20.48	1.076	0.157
Cholesterol (mg/dL)	115.47	100.39	104.81	8.720	0.476
High density lipoproteins (mg/dL)	58.47	53.24	54.58	4.096	0.654
Low density lipoproteins (mg/dL)	25.16	19.37	22.38	4.095	0.618
Triglyceride (mg/dL)	30.94^{a}	29.13^{a}	23.67^{b}	1.640	0.022
Albumin (g/dL)	2.61	2.58	2.55	0.839	0.899
Total protein (g/L)	60.04	59.85	61.92	1.622	0.621
Enzymes activities					
Aspartate transaminase (U/L)	59.57	58.48	55.69	5.314	0.869
Alanine aminotransferase (U/L)	19.99	18.88	18.12	1.746	0.702
Hormones					
T_3 (ng/dL)	172.60	168.40	177.80	14.75	0.904
T_4 (mg/dL)	4.01	3.48	3.96	0.413	0.624
Insulin (mIU/mL)	17.60^{a}	30.40^{b}	27.80^{ab}	3.930	0.090
Growth hormone (mIU/mL)	0.28	0.25	0.31	0.037	0.496
Cortisol (nmol/L)	11.40	10.40	10.20	3.403	0.965

PEG: polyethylene glycol and G-bind: activated sodium bentonite.
The means within the same row with at least one common letter, do not have significant difference (P>0.05).
SEM: standard error of the means.

Results showed that there were no significant differences among treatments for hair fiber characteristics (P≥0.05) (Table 7). In this study, PEG and activated sodium bentonite at 1.0% of DM were supplemented to the basal diet of goats containing 30% diet PBP (% DM) as tanniniferous roughage.

PEG supplementation did not affect DM intake and ADG of experimental groups, which is consistent with other authors who reported no performance improvement by PEG supplementation (Bhatta *et al*. 2002).

Reduced feed intake and nutrient digestibility by dietary CT have been reported in the literature (Frutos *et al*. 2004),

but also it has been reported that feeding PBP in the diet of fattening lambs (Norouzian and Ghiasi, 2012) and dairy goats (Sedighi-Vesagh *et al*. 2015) up to 30% had no adverse effects on DMI, ADG, feed conversion rate (FCR). Effects of CT on animal performance are considered to be dependent on the level of CT in the diet (Peng *et al*. 2016). Supplementation of PEG400 to the diets of sheep based on 40% concentrate and 60% purple prairie clover (as a tannin-rich diet), improved significantly nutrient digestibility and blood glucose and urea but did not affect growth performance (Peng *et al*. 2016). On the contrary, others working with penned sheep (Ghandour *et al*. 2014) and goats (Brown and Ng'ambi, 2017) restricted in the variety of feeds reported increased DM intake and improved animal performance (Decandia *et al*. 2000; Motubatse *et al*. 2008) by PEG400 supplementation. Silanikove *et al*. (1996b) found a significant increase in the DM intake of goats dosed with PEG and fed with tannin-containing roughage. However, their goats were fed only by foliage from tanniniferous species, without supplementation of concentrate.

Ghandour *et al*. (2014) by supplementation of growing lambs diet with PEG and sodium bentonite reported that adding these supplements to Acacia diet could be used to increase feed intake utilization as result to tannins deactivation. Gouda *et al*. (2019) by addition of bentonite in goat's diet that was naturally contaminated with aflatoxins reported that the digestibility of DM, organic matter, CP, EE, NDF, and ADF increased, although it did not affect nutrient intake. Besides, their study indicated the goats that received the bentonite treatment had greater ruminal ammonia-N and total volatile fatty acid in the rumen. According to our results, Aydin *et al*. (2020) by supplementation of sodium bentonite to the lamb's diet found that there was no difference among groups in terms of live weight gain, ADG, feed intake, and FCR.

It has been suggested that PEG has the potential to increase the productivity of goat feeding in tannin-rich environments (Decandia *et al*. 2000). Ben Salem *et al*. (2005) showed that PEG4000 inactivated CT, thus improving microbial protein synthesis and growth of sheep. PEG also increased significantly DM intake when provided at 20 g/day to goats fed solely by lentisk leaves (as tannin-rich roughage) (Silanikove *et al*. 1996a).

The lack of an alteration in DMI and body weight gain (BWG) in our trial may be interpreted by the low concentration of CT in the basal diet. Also, it can be related to the same level of tannin content in all treatments in the present study. Besides, the ability of goats to consume higher amounts of tannins is higher than other ruminants (Silanikove *et al*. 1996b).

Goats are more resistant to tannin toxicity than cattle and sheep because the secretion of proline-rich proteins is higher during eating in goats than that in sheep and cattle (Rivero *et al*. 2016). The parotid saliva of goats is also rich in proline, glutamine, and glycine which have a high tannin-binding capacity, by enhancing the affinity of proteins to tannins (Olafadehan, 2011). Moreover, rumen microbes are capable of degrading some tannin. Rumen microorganisms at low tannin levels can metabolize tannins or remain active in a high tannin environment and overcome their detrimental effects, which in turn improves animal performance (Makkar, 2003).

G-bind supplementation also did not affect performance parameters among treatment groups. Jerónimo *et al*. (2010) reported that DMI and daily weight gain was not affected by adding bentonite in diets of finishing lambs. Azadbakht *et al*. (2017) reported that supplementation of lead-exposed lambs diet with 1.5% bentonite significantly improved daily weight gain and feed conversion ratio.

In the present study, ruminal pH was not different among treatments. Although the rumen volatile fatty acids were not measured in the present study, this stability in ruminal pH may be attributed to the lack of change in the primary ruminal fluid VFA due to dietary treatments. Condensed tannins in the present study were 1.93% of the diet DM. It has been reported that concentrations of CT below 5% of DM in the diet do not have important effects on ruminal fermentation (Barry and McNabb, 1999). Sedighi-Vesagh *et al*. (2015) by adding PEG to a diet containing 32% PBP resulted that, DMI was not affected by treatments but CP digestibility significantly increased by PEG addition to the diet. In another study, Ghaffari *et al*. (2013) investigated the effect of the addition of PEG to a diet containing 30% PBP and reported that there were no differences between treatments for ruminal pH, but DMI improved by using PEG. Alipanahi *et al*. (2019) reported that adding PEG improved CP digestibility, but did not affect ruminal pH in goats fed with an oak acorn ration containing hydrolyzable tannins. Xie *et al*. (2021) by supplementation of PEG to steer ration containing sorghum tannins showed that ruminal pH and CP digestibility increased by PEG addition. Seoni *et al*. (2021) found that feeding lambs diets containing birdfoot trefoil with PEG caused an increase in the DMI and a reduction in the DM digestibility. Also, they reported that urinary and total N excretion increased in lambs fed diets with PEG. In another study, Akbag (2021) investigated the potential nutritive value of Anagyris foetida shrub as tanniniferous forage for goats and concluded that PEG can be used to enhance rumen fermentation conditions during the browsing of *Anagyris foetida*.

Table 6 Effect of different treatments on mineral content (mg 100 g^{-1}) of latissimus dorsi muscle from Saanen goats

Parameters	Treatments			SEM	P-value
	Control	PEG	G-bind		
Calcium	4.31[b]	4.79[b]	7.33[a]	0.765	0.044
Copper	0.54	0.69	0.61	0.099	0.618
Zinc	0.53[b]	1.26[a]	0.61[b]	0.181	0.041
Iron	0.475[c]	2.00[a]	1.68[b]	0.089	0.0001

PEG: polyethylene glycol and G-bind: activated sodium bentonite.
The means within the same row with at least one common letter, do not have significant difference (P>0.05).
SEM: standard error of the means.

Table 7 Effect of different treatments on hair fiber characteristics parameters of Saanen goats

Parameters	Treatments			SEM	P-value
	Control	PEG	G-bind		
Fiber diameter (µm)	130.80	136.95	137.52	2.542	0.263
Coefficient of variation of fiber diameter	24.05	18.71	19.52	2.722	0.340
Fiber diameter distribution (µm)	31.37	25.68	30.38	2.602	0.379
Washing efficiency (%)	69.24	75.63	78.91	2.171	0.107
Fiber length (cm)	1.08	1.31	1.22	0.844	0.212

PEG: polyethylene glycol and G-bind: activated sodium bentonite.
SEM: standard error of the means.

Blood hematological and biochemical parameters

The results showed that there was no significant difference among experimental groups in hematological parameters except monocyte count. The PEG and G-bind supplementation significantly (P<0.05) increased monocyte count. None of the measured blood serum metabolites except TG were affected by dietary treatments. In previous work, it has been indicated that dietary inclusion of PBP (15% DM) had no effects on serum metabolites of early lactation cows (Rezaeenia *et al*. 2012).

Xie *et al*. (2021) by supplementation of PEG to steer ration containing sorghum tannins reported that adding PEG to basal diet linearly increased plasma globulin and total protein concentration, that it could be attributed to the increase in CP digestibility. Alipanahi *et al*. (2019) indicated that plasma concentration of globulin, urea, TG, total protein, and albumins were not influenced by the inclusion of oak and PEG in the diet.

The value of packed cell volume (PCV) is a rapid screening technique for seeking anemia and is used as an indicator of animal hydration (Smith, 2007). In the present study, the PCV values in the treatment groups were within reference values for goats (22-38%) and cannot be considered as an indicator of anemia or dehydration.

The white blood cell (WBC) count at the present study was 111 × 10^2 µL, 130 × 10^2 µL, and 154 × 10^2 µL in G-bind, PEG, and control group, respectively. Therefore, according to the normal range of WBC reported for goats (40-130×10^2 µL), this index only in the control group was beyond the normal range. In other words, it might be considered that feeding a tanniniferous diet increased the WBC count of animals, and dietary supplementation of PEG or G-bind perhaps rendered enhanced WBC count into the normal range. However, contrary to this inference, Solaiman *et al*. (2010) indicated decreased WBC counts with increasing tannin intake in the diet.

Monocytes are essential for the immune system as they are precursors of macrophages and lymphocytes essential for humoral and cell-mediated immunity responses (Mahgoub *et al*. 2008b). In the present study, monocytes count increased significantly by dietary addition of PEG and G-bind compared to control treatment. This result is consistent with some previous literature that reported that monocyte counts decreased for the goats (Olafadehan, 2011) and sheep (Mahgoub *et al*. 2008b) were fed with a tannin-containing forage diet. Tannin composition and tannin concentration in the diet may play a role in how a tannin-containing diet affects blood monocyte concentrations. It has been reported that orally supplementation of 30 mL *S. babylonica* extract containing tannins and plant second metabolites in goats increased significantly eosinophils number in blood samples compared to control, but did not affect monocytes and other hematological and biochemical parameters (Smith, 2007).

Mean corpuscular hemoglobin concentration (MCHC) is important for the diagnosis of anemia in most animals; therefore being this parameter in normal ranges (30-36 g/dL) in all experimental groups suggested the absence of microcytic hypochromic anemia (Solaiman *et al*. 2010). In the present study, PCV, Hb, and lymphocyte count were not affected by dietary treatments and were within the established ranges reported for healthy goats (Sirois, 1995). In a previous study, the lower PCV and red blood cell (RBC) for goats (Olafadehan, 2011) and sheep (Mahgoub *et al*. 2008b) fed a sole tannin-rich forage than that fed sole tannin-free forage was attributed to the presence of phenols

and CT in the consumed tanniniferous diet, that have been reported to have anti-nutritional factors.

In the present study, there was no consistent effect of PEG and G-bind supplementation on the serum glucose concentration of the goats. Serum glucose at all experimental groups (Table 5) was within the normal range of 50-75 mg/dL reported for healthy goats (Mohammed *et al*. 2016). These findings are similar to the report of Ben Salem *et al*. (2005), Rezaeenia *et al*. (2012), and Azadbakht *et al*. (2017). Gouda *et al*. (2019) by addition of bentonite in goat's diet that was naturally contaminated with aflatoxins found that this additive supplementation increased the concentration of plasma glucose that was related to toxin absorption properties of bentonite. Aydin *et al*. (2020) by using sodium bentonite to the lamb's diet indicated that this additive did not influence plasma concentrations of glutathione, albumin, globulin, and total protein.

No significant difference in blood glucose among experimental groups may indicate that at the present study the dietary energy was sufficiently utilized for animal growth and the intake of tannins was lower than the tolerable intake of 1.1-2.7 g/kg body mass on goats (Silanikove *et al*. 1996b). The blood sugar-decreasing effect of CT was reported for CT-containing forages fed to sheep (Mahgoub *et al*. 2008b). The mechanism by which CT decreases blood glucose concentration is not clear. However, a decrease in the molar proportion of propionate in the rumen and devaluation of feed consumed by CT (Mahgoub *et al*. 2008a) may in part account for the lower blood glucose concentration (Peng *et al*. 2016).

Changes in serum urea N may be an indicator of kidney damage. A lack of increase in this metabolite above the normal values but below the normal range suggests that necrotic damage to the kidney did not occur. The blood urea nitrogen (BUN) concentration in ruminants has been used as an indicator of excess N consumption relative to energy. In ruminants, BUN can be influenced by dietary N-to-energy ratio, level of forage intake, and protein degradability in the rumen (Hammond *et al*. 1994). Adding PEG to the PBP in the present study had no significant effect on BUN following Nishida *et al*. (2006) who stated that green tea waste, which contained a large amount of tannin, did not affect nitrogen availability (rumen NH_3-N concentration and BUN) to Holstein steers. In the present study, serum urea level in all experimental groups was below the normal established range of 25-60 mg/dL for healthy goats (Mohammed *et al*. 2016). Moreover, total protein content in the sera (5.98 g/dL) of the control group was slightly below of normal range (6-7.5 g/dL) (Sirois, 1995; Mohammed *et al*. 2016), which may be attributable to CT effects in the diet containing mere PBP. It has been suggested that the presence of CT in the diet reduce BUN by the formation of tannin–protein complexes in the rumen led to a reduction in ruminal protein degradation and ruminal NH_3–N concentration and subsequently reduced ammonia transfer into the blood for the conversion of urea in the liver (Silanikove *et al*. 1996a; Ben Salem *et al*. 2005). Ben Salem *et al*. (2005) reported an increase of serum urea concentrations when 10 g of PEG4000 was dietary supplemented to neutralize the tannins of *Quercus coccifera* in goats. These authors ascribed this to increased diet digestibility and increased absorption of amino acids from the gut due to the PEG effect (Silanikove *et al*. 1996a). High levels of dietary protein or species differences may be involved in this dispute results. In a previous study, little or no influence of sodium bentonite on ruminal fermentation and plasma urea nitrogen (Ivan *et al*. 1992b). Among the measured hormones in the blood, only insulin levels increased significantly in the PEG group compared to the control, while the glucose concentration did not change between these two groups. It has been suggested that CT when directly acts on adipose cells, enhances insulin receptors of fat cells (Klein *et al*. 2007). It is possible that the binding of PEG400 to CT in the gut inhibits their absorption and thereby induced partial insulin insensitivity in goats.

Serum TG concentration in the group supplemented by sodium bentonite decreased significantly compared to other treatments (Table 5). This may be due to the absorptive and activating effects of bentonite on lipase enzyme in the post-ruminal gastrointestinal tract (Ghandour *et al*. 2014). This result is inconsistent with others who reported sodium bentonite had no significant effect on blood lipid profile (Azadbakht *et al*. 2017).

There was no significant difference among goats given the three dietary treatments in total cholesterol, high density lipoprotein (HDL), and low density lipoprotein (LDL). Similarly, Eruden *et al*. (2004) reported no difference in plasma concentrations of TG, total cholesterol, LDL-cholesterol, by feeding green tea waste silage with or without PEG to Holstein steers.

The normal ranges for alanine aminotransferase (ALT) and aspartate transaminase (AST) are 7-24 U/L and 43-132 U/L, respectively in goats (Sirois, 1995). The fact that none of these hepatic enzymes in the present study differed among experimental groups and both of them fell within the normal ranges for goats (inconsistency with the results of Silanikove *et al*. 1996b; Mahgoub *et al*. 2008b; Olafadehan 2011) suggest that no damage to the liver occurred.

It was evident that the deactivation of tannins in PBP by using PEG or activated sodium bentonite affected the mineral content of latissimus dorsi muscle of the experimental animals as illustrated in Table (7). Dietary inclusion of PEG significantly increased Zn and Fe content of latissimus dorsi muscle of goats. It has been reported that the mineral

content of muscle tissues in small ruminants can vary considerably, and nutrition is an important player in determining mineral concentration in muscle tissue (Reykdal and Thorlacius, 2001). Polyphenolic compounds and tannins are capable of forming effective cross-links with minerals and inhibit the absorption of some minerals in the gastrointestinal tract (Henry *et al*. 1992). Therefore, it is possible that PEG, by neutralizing the inhibitory effect of tannins on mineral uptake, has increased the uptake of these minerals and increased their density in muscle. On the contrary, it was shown that major minerals (Ca, Zn, Cu, Fe) except Mg were not affected by feeding a sole tannin-rich forage to goats (Olafadehan, 2011) and absorption of minerals from the gastrointestinal tract, particularly Ca, P and Mg, and mineral content of body organs was not disturbed by feeding tannin-rich roughages (Silanikove *et al*. 1996b). Also, feeding PBP in a diet of fattening lambs up to 30% had no adverse effects on the mineral content of lamb meat, and this attributed to low chelation properties of absorbed tannins from PBP (Norouzian and Ghiasi, 2012). Calcium and Fe content in latissimus dorsi muscle of the G-bind group was significantly higher than control (Table 6). Ivan *et al*. (1992a) reported that sodium bentonite reduced the solubilities of some cations, but not Fe and Mn. In a previous study, it has shown that the mineral content of body organs is affected by dietary sodium bentonite, given its binding qualities (Fenn and Leng, 1989).

According to Table (7), PEG and G-bind inclusion in the basal diet had no significant effect on hair growth. The effectiveness of bentonite on hair growth in the present study may be due to the constant availability of feed to animals (Fenn and Leng, 1989). Parallel results were obtained by Walz *et al*. (1998) who also indicated supplementation of high-concentrate diets with 0.75% sodium bentonite has no beneficial effect on wool growth of growing lambs. This was explained by the higher nutrient demand of lambs for body growth than for wool growth so that, young lambs may partition nutrients toward body growth rather than wool growth to a greater extent than do adult sheep.

Seied Moemen (2003) indicated that feeding 10, 20, and 30% DM PBP to Raini goats had no adverse effect on animal performance; even feeding 10% DM PBP to goats increased production and strength of goat hair.

Wool growth seems to be stimulated by sodium bentonite when sheep are fed low-energy diets (Cobon *et al*. 1992). Supplementation of PEG4000 to the diets of sheep based on 40% concentrate and 60% purple prairie clover (as tannin-rich forage), did not affect wool yield and wool quality (Peng *et al*. 2016). Wool production is strongly correlated with feed intake (Rangel and Gardiner, 2011). Therefore, the similar feeding value of the experimental diets potentially explains the similar hair growth and fiber characteristics of goats among the three dietary treatments.

CONCLUSION

The present results indicated that the addition of PEG and G-bind to a diet containing 30% PBP had some beneficial effects on blood parameters as increasing the monocyte count using both of these additives and decreasing the TG concentration, only by G-bind addition to the diet. Besides, G-bind significantly improved the Ca and Fe content of latissimus dorsi muscle of goats compared to the control. Also, dietary supplementation of PEG significantly increased Fe and Zn content of muscle in comparison to the control group. In conclusion, it seems that activated sodium bentonite with the low price has some ability like PEG as tannin-deactivation material in diets containing 30% DM pistachio by-products for feeding to goats.

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