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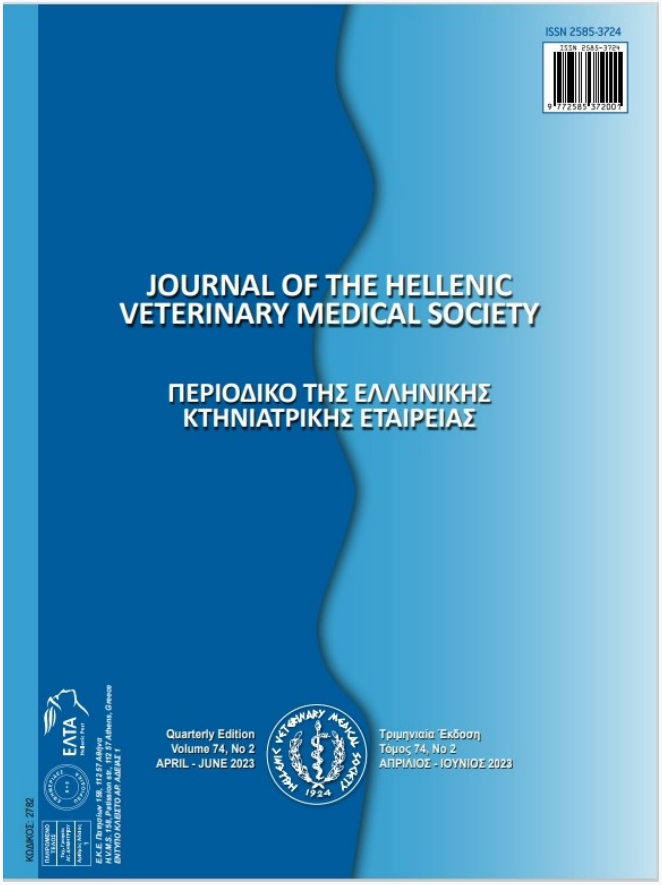

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Effect of Solid State Fermentation with Whey on Nutrient Composition of Pomegranate Peel Supplemented Sunflower Meal

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ABSTRACT: The aim of the study is to improve the nutritional value of sunflower meal by solid state fermentation (SSF) using agricultural wastes such as whey and pomegranate peel.
Experiment was conducted with six groups. First group SM (sunflower meal) + TW (tap water), the second group SM + W (whey), the third group SM + W + 0.5% PP (pomegranate peel), the fourth group SM + W + 1% PP, the fifth group SM + W + 1.5% PP and the sixth group SM + W + 2% PP, respectively. The mixtures were prepared to complete 100 grams of sunflower meal with 0, 0,5, 1,0, 1,5 and 2,0 grams of pomegranate peel (PP), respectively. Each group consisted of eight replicates. Prepared mixtures were placed in 500 ml erlenmayers and 120 ml tap water was added to the mixing of first group and 120 ml whey was added in other groups and then mixed homogeneously. Four of the erlenmayers prepared for each group without being fermented were dried at room temperature. The remaining erlenmayers were fermented in 32°C±2 for 48 hours. Dry matter, crude protein, ether extract, crude ash, crude fiber, phytic acid ratios and phytase activities, antioxidant activities and yeast (*Saccharomyces cerevisiae*) numbers were determined in fermented and non-fermented feed samples.
As a result of solid state fermentation with whey by adding pomegranate peel to sunflower meal, crude protein ratio, antioxidant activity and yeast count increased, and phytic acid ratio decreased. In conclusion, sunflower meal with functional properties was obtained. In addition, wastes such as whey and pomegranate peel were used as feed and environmental pollution was prevented.

***Keywords*:** Sunflower meal; pomegranate peel; whey; solid state fermentation; functional meal

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INTRODUCTION

Soybean meal, which forms the basis of vegetable protein sources in poultry, is a high-cost feed. Therefore, the use of other vegetable protein sources in poultry nutrition instead of soybean meal has been the subject of research. Researches in recent years have shown that sunflower seed meal has started to replace soybean meal (Ceylan, 2012).

Sunflower seed meal has a very important place in animal nutrition since it is cheap, nutritious and high in crude protein (Battaloğlu, 2007). However, due to the high cellulose it contains, sunflower seed meal has an extremely low digestibility rate and feed value (Akyıldız, 1986).

Various methods have been tried to increase the rate of use of poultry rations of sunflower seed meal. One of these methods is solid state fermentation.

Solid state fermentation (SSF) is mostly defined as the growth and development activity of microorganism cultures in the environment where there is little or no humidity (Pandey et al., 2008).

Functional properties can be provided to feed ingredients by SSF method (Tosun, 2017). Soltan et al. (2015) reported that the crude protein, crude oil and crude ash values of the sunflower seed meal, which it subjected to solid state fermentation with *Saccharomyces cerevisiae*, increased significantly, and the crude cellulose, phytic acid and trypsin inhibitor decreased. In addition, it was reported in the same study that fermented sunflower meal can be substituted for soybean meal at a rate of 25% for fish rations. In another study, it was found that crude protein, free amino acid and crude ash ratios of sunflower seed meal fermented with *Bacillus subtulis* increased significantly, while the ratios of phytic acid and crude cellulose decreased (Uysal, 2017).

The correct management of waste products released in the agriculture and food industry can provide cheap and suitable feed materials for poultry animals without competion for human food. Therefore, waste products such as whey and pomegranate peel were used in the improvement of sunflower meal.

Pomegranate (*Punica granatum* L.), which is a tropical fruit, is grown in abundance in our country and it was produced around 537,847 tons (Anonymous, 2018) in 2018. Pomegranate, whose peel consists of approximately 48% of its total weight (Zarei et al., 2011), shows high levels of antioxidant and antitumor activity thanks to the phenolic substances it contains (Yalınca and Yıldız, 2010). It has been suggested that pomegranate peel or pulp, which becomes waste after processing, can be used as an alternative feed source (Sarıca, 2011).

Whey is an important dairy by-product that contains varying levels of lactose, fat, mineral substances and vitamins with serum proteins such as lactoalbumin and lactoglobulin (Kurt, 1990). When it is thrown into the environment without any treatment, it causes environmental pollution and also causes the loss of high-value nutrients. The use of whey, which is also very useful in terms of animal nutrition, in various ways is of great economic importance.

In this study, it was aimed to increase the nutritional value as a result of solid state fermentation with whey by adding pomegranate peel at different rates to sunflower seed meal.

MATERIALS AND METHODS

In this study, it was aimed to determine the effect of solid-state fermentation (SSF) with whey (W) on the nutrient composition of pomegranate peel (PP) supplemented sunflower meal (SM). Experiment was conducted with six groups such as the first group SM + TW (tap water), the second group SM + W, the third group SM + W + 0.5% PP, the fourth group SM + W + 1% PP, the fifth group SM + W + 1.5% PP and the sixth group SM + W + 2% PP, respectively. The mixtures were prepared to complete 100 grams of sunflower meal with 0, 0,5, 1,0, 1,5 and 2,0 grams of pomegranate peel (PP), respectively. Each group consisted of eight replicates. The prepared mixtures were placed in 500 ml of Erlenmeyer flask. 120 ml of tap water was added to the mixture in the first group and 120 ml of whey was added to the other groups and mixed homogeneously. The pH values of the prepared mixtures were measured and with the help of citric acid, it was ensured that the values were between 4-4.5, which is ideal for the development of yeasts. Four of the erlenmeyers prepared for each group were dried at room temperature without being fermented. The remaining four erlenmeyer flasks were fermented at 32°C±2 for 48 hours.

In fermented and unfermented feed samples, dry matter, crude protein, ether extract, crude ash, crude fiber (Kutlu, 2008), antioxidant activities (Blois, 1958), phytic acid ratios (Raheja et al., 1973), phytase activities and yeast numbers (Halkman, 2007) was determined.

Statistical Analyses

The analyzes of the data were made using the General Linear Model procedure and the SPSS 17.0 package program. Significance checks of means found significant between groups were determined by Duncan Multiple Comparison Test (Düzgüneş et al., 1983). The differences between the nutritional values of the feeds before and after fermentation were determined by t test.

RESULTS AND DISCUSSION

When Table 1. was examined, it was found that there was no statistically significant difference in dry matter values between the groups before fermentation. However, there were significant differences (P<0.05) between the groups in terms of dry matter after fermentation and the highest dry matter ratio was found in the SM + W + 1.5% PP group. When the dry matter values of the feeds were examined before and after fermentation, it was seen that there was no difference in dry matter ratios depending on the fermentation.

Uysal (2017) stated that there is a significant decrease in the dry matter rate due to fermentation time in the sunflower seed meal, which it subjected to solid state fermentation with *Bacillus subtilis*.

It was determined that there was no significant difference between the groups before fermentation in terms of crude protein value, but the difference was significant after fermentation and the highest crude protein value was found in the SM + W + 0.5% PP group (increased by 15.11%). In the study, it was found that the crude protein value of the mixture increased after fermentation in all groups in which whey was added compared to before fermentation (Table 1).

In the research, a significant increase in the yeast count of the feed mixtures was observed depending on the fermentation. The increase in the crude protein ratio of the feeds was associated with the increased yeast count. Considering that the dry matter of the yeast consists of 45-60% of the raw protein (Pamir, 1985), the reason for this increase in the protein value of the feed is revealed. Similarly, Soltan et al. (2015) reported that the crude protein content of the sunflower seed meal, which they had fermentation with yeast, increased from 30.70% to 38.40%. Many studies have reported that crude protein values increase as a result of fermentation of different feed sources with yeast or other microorganizms (Moore et al., 2007; Frias et al., 2008; Kaur et al., 2009; Rashad et al., 2011; Yaşar, 2014; Hassaan et al., 2015).

It was determined that the crude ash values of the whey added feed samples were considerably higher than the tap water added group (P<0.01). After fermentation, there was no significant difference between the groups in terms of crude ash rates. When the effect of fermentation was examined, it was observed that the crude ash values increased significantly (P <0.01) after fermentation in the SM + W + 1% PP, SM + W + 1.5% PP and SM + W + 2% PP groups. The increase in the crude ash ratio after fermentation showed parallelism with the increase in the pomegranate peel level.

Yeasts with an average of 6-9% crude ash can be shown as the reason for the increase in the crude ash rate of feed due to fermentation (Pamir, 1985). Because it has been determined that the number of yeasts in feed has increased significantly due to fermentation (Table 2).

Table 1. Dry matter, crude protein, ether extract, crude ash and crude fiber rate of fermented and non-fermented feeds.

GROUPS	Dry Matter %		Crude Ash %		Crude Protein %		Ether Extract %		Crude Fiber %	
	B.F	A.F	B.F	A.F	B.F	A.F	B.F	A.F	B.F	A.F
SM+TW	88,56	88,28^{abc}	6,03^{b}	7,32	25,86	25,97^{b}	0,70^{b}	0,58^{c}	25,45	25,76
SM+W	88,75	87,77^{abc}	7,24^{a}	7,48	26,09B	27,83^{b}A*	1,07^{a}	0,94^{ab}	25,58	25,53
SM + W + 0.5% PP	88,48	86,99^{c}	6,60^{a}	7,07	25,59B	30,57^{aA}**	1,07^{a}	0,96^{ab}	25,31	25,69
SM + W + 1% PP	87,85	86,92^{c}	6,48^{ab}B	8,10**A	26,16B	26,98^{b}A*	1,23^{a}	1,04^{a}	26,09	25,66
SM + W + 1.5% PP	88,79	89,50^{a}	6,41^{ab}B	7,97A**	25,30B	28,09^{b}A*	1,15^{a}	0,89^{ab}	25,14	25,62
SM + W + 2% PP	88,06	87,22^{bc}	6,89^{a}B	8,08A**	25,97B	27,23^{b}A*	1,17^{a}	1,10^{a}	25,95	26,26
SEM	0,19	0,27	0,15	0,20	0,20	0,37	0,05	0,05	0,20	0,12
P	ns	*	**	ns	ns	**	*	*	ns	ns

a-c: The means shown by different letters in the same column are different from each other. A-B: Different letters show that the averages of each parameter differ from each other in two different fermentation times. A.F: After fermentation, B.F: Before fermentation SM: Sunflower meal TW: Tap water, W:Whey, PP: Pomegranate peel, *: P<0.05, **: P<0.01 ns: not significant.

Table 2. Phytase activities, phytic acid ratios, antioxidant activities and yeast numbers of fermented and non-fermented feeds.

GROUPS	Phytase Activities (IU/g KM)		Phytic Acid Ratios (%)		Antioxidant Activities (%)		Yeast (*Saccharomyces cerevisiae*) Numbers %	
	B.F	A.F	B.F	A.F	B.F	A.F	B.F	A.F
SM+TW	0.436^{ab}B	0.472^{c}A**	0.515 A	0.386^{a}B*	53.69^{d}B	61.21^{c}A	4.77 x 10^{3}B	5.72 x 10^{7}A**
SM+W	0.385^{c}B	0.540^{b}A	0.525A	0.348^{b}B*	59.91^{bc}B	73.17^{b}A	6.00 x 10^{3}B	6.52 x 10^{7}A**
SM + W + 0.5% PP	0.399^{c}B	0.533^{ab}A**	0.520A	0.349^{b}B**	63.51^{abc}	68.77^{bc}	4.85 x 10^{3}B	6.10 x 10^{7}A**
SM + W + 1% PP	0.455^{a}B	0.577^{ab}A**	0.520A	0.361^{b}B**	63.89^{abc}B	74.17^{b}A	5.02 x 10^{3}B	6.19 x 10^{7}A**
SM + W + 1.5% PP	0.411^{bc}B	0.575^{ab}A**	0.525A	0.312^{c}B**	66.21^{ab}B	86.54^{a}A	5.08 x 10^{3} B	6.23 x 10^{7}A**
SM + W + 2% PP	0.453^{a}B	0.602^{a}A**	0.535A	0.358^{c}B**	69.54^{a}B	85.19^{a}A	5.23 x 10^{3}B	6.12 x 10^{7}A**
SEM	0.009	0.018	0.009	0.017	1.37	2.35	0.028	0.031
P	**	**	ns	**	**	**	ns	ns

a-c: The means shown by different letters in the same column are different from each other. A-B: Different letters show that the averages of each parameter differ from each other in two different fermentation times. A.F: After fermentation, B.F: Before fermentation SM: Sunflower meal TW: Tap water, W:Whey, PP: Pomegranate peel, *: P<0.05, **: P<0.01 ns: not significant.

Similar to this study, Soltan et al. (2015) reported that the crude ash rate of sunflower seed meal, which they undergo solid state fermentation with *Saccharomyces cerevisiae*, increased from 6.90% to 7.80%. Hassaan et al. (2015) found that the amount of crude ash of soybeans, which it fermented with *Saccharomyces cerevisiae*, increased from 61 g/kg to 71 g/kg.

When Table 2. was examined, it was determined that there was a significant (P <0.05) difference between ether extracte values between the groups before fermentation. The lowest value was found in the SM + TW group. After fermentation, there was a significant (P <0.05) difference between the groups in terms of ether extracte values, the lowest value was observed in the SM+ TP group, the highest values in the SM + W+ 1% PP and SM + W + 2% PP groups. It was thought that the increase in ether extract ratio both before and after fermentation was caused by whey.

Considering the effect of fermentation, it was determined that the extract ratios of the feeds decreased slightly after fermentation, but this change was not significant. Unlike the current study, Soltan et al. (2015) found that the eter extracte value of the sunflower meal that they fermented with yeast increased. However, Hassaan et al. (2015) determined that the eter extracte rate of soybean meal, which they fermented with yeasts, decreased significantly. Baran (2017) reported that the ether extract ratios of barley, wheat and oat fermented with *Lactobacillus salyarius* changed significantly, the ether extract ratio of wheat and oat increased while the ether extracte ratio of barley decreased.

It was found that there was no change in crude fiber ratios of feeds either before or after fermentation. Fermentation did not have a positive effect on crude fiber.

It has been reported that the rate of crude fiber decreases up to 45% as a result of fermentation of soybean by-product with different yeast strains, and this decrease decreases to 7.38% in fermentation with *Saccharomyces cerevisiae* (Rashad et al., 2011). Researchers have shown the enzymes that break down the cellulose/hemicellulose secreted by yeasts as the reason for this decrease in the crude cellulose ratio (Lateef et al., 2008).

On the other hand, Soltan et al. (2015) reported that the crude fiber value of sunflower seed meal fermented with *Saccharomyces cerevisiae* decreased from 21.6 g/100 g to 7.30 g/100 g. Similarly, Hassaan et al. (2015) stated that the crude fiber value of soybean meal fermented with *Saccharomyces cerevisiae* decreased by approximately 50%. Researchers have tried to explain the situation by arguing that various enzymes are secreted during solid state fermentation that disrupt the structure of crude fiber and complex polysaccharides in the environment.

In this study, it was determined that the crude fiber values did not change depending on the effects of treatments and fermentation. The reason for this can be explained by the absence of any external yeast addition for fermentation, and the fact that the number of yeasts that naturally increased during fermentation did not reach the level that could dissolve the crude fiber.

It was observed that there was a significant (P<0.01) difference between the groups before fermentation in terms of phytase activity, and the highest

activity value was determined in the SM + W + 1% PP and SM + W + 2% PP groups. It was determined that the phytase activity of the mixtures to which pomegranate peel was added after fermentation increased significantly, and the highest value was in the SM + W + 2% PP group (P<0.01). When the effect of fermentation was examined, it was found that phytase activity increased significantly in fermented groups compared to unfermented ones. Depending on the fermentation, an increase in phytase activity between 8.25% and 40.25% was observed (Table 2).

When Table 2. was examined, it was determined that there was no significant difference between the groups in terms of phytic acid ratios of the feeds before fermentation. However, very significant (P<0.01) differences were determined in terms of phytic acid between the groups after fermentation. The lowest phytic acid ratio was observed in the SM + W + 1.5% PP group. Considering the effect of fermentation, it was observed that the phytic acid ratio decreased significantly in all feeds after fermentation, and the largest change rate was 40.6% in the SM + W + 1.5% PP group.

Phytic acid, an organic compound, is a storage form of phosphorus in legumes and grains. Most of the phosphorus in grains and oilseeds is phytate, and 28.2% of phytate is reported to be found in the form of phytin phosphorus (Sauvant et al., 2004).

Since there is no phytase enzyme in the digestive system of poultry, they cannot benefit enough from the phosphorus in these plants. Phytase, an enzyme that catalyzes the degradation of phytate, is present in different amounts in wheat, rye, barley and triticale, but is not found in oats, maize and untreated seeds (Boisen, 1987; Eeckhout and De Paepe, 1994).

Since poultry cannot digest the phytate in the feed to a large extent, phytate accumulation occurs in the fecal material, and as a result, phosphorus pollution occurs in areas where intensive livestock is made (Maenz and Classen, 1998). In this study, it was determined that phytase activity increased and the phytic acid ratio decreased significantly as a result of fermentation of SM with whey. Similarly, Soltan et al. (2015) found that the phytic acid value of sunflower seed meal fermented with yeast decreased from 0.363 g/100 g to 131 g/100 g. Kemme and Jongbloed (1993) observed that the digestibility of phosphorus increased when a diet supplemented with phytase was soaked in water at room temperature for 8 to 15 hours. Näsi et al. (1995) found that a diet soaked with whey for 3 hours at 40 °C significantly increased P absorption in pigs. Similarly, Skoglund et al. (1997) reported that soaking a diet for 9 hours at room temperature reduced its phytate content by 45%. Carlson and Poulsen (2003) stated that phytate levels in barley and wheat fermented by soaking at 10, 20 and 30 °C decreased significantly due to fermentation. Uysal (2017) determined that the phytic acid ratio decreased significantly in sunflower seed meal, which was subjected to solid state fermentation with *Bacillus subtulis*.

It was determined that there was a very significant (P<0.01) difference between the groups before fermentation in terms of % DPPH. The lowest DPPH rate was determined in the SM + TW group, and the highest rate was determined in the SM + W + 2% PP group. After fermentation, the lowest rate was found in the SM + W group, and the highest values were found in the SM + W + 1.5% PP and SM + W + 2% PP groups. Except for the SM + W + 0.5% PP group, it was observed that the % DPPH values increased significantly due to fermentation in all groups and the highest proportional increase was in the SM + W + 1.5% PP group.

Sunflower (*Helianthus annuus*), an important oilseed plant, is used in human nutrition at a certain level. The pulp that remains after the oil is separated is mostly used as animal feed. Many studies have reported that sunflower seed meal has high antioxidant activity. The high antioxidant properties of various sunflower polyphenols such as caffeic, chlorogenic and ferulic acid have been proven by studies. By-products, such as husk, produced during sunflower oil production are a valuable source of phenolic compounds that can be recovered and used as natural antioxidants (Weisz et al., 2009). Wanjari and Waghmare (2009) determined the antioxidant activity of sunflower seed meal extracted with ethanol as 3264,1%. As a result of the present study, it was determined that the antioxidant activity of SM, which was not subjected to any treatment, was 55.56%. It was observed that the antioxidant activity was highest in the treatment groups in which 1.5% and 2% pomegranate peel were added. The DPPH inhibition of the pomegranate peel used in the experiment was determined as 80%.

Pomegranate, which is highly produced in our country, has very high antioxidant and antitumor activity properties due to the many phenolic substances it contains. As a result of the researches, it has been

reported that the polyphenolic compounds in the structure of the pomegranate peels can be used as natural antioxidants (Gil et al., 2000; Singh et al., 2002; Li et al., 2006; Shabtay et al., 2008). It has been stated by many researchers (Murthy et al., 2002; Shabtay et al., 2008) that the polyphenols in the pomegranate peel significantly prevent lipidoxidation. In previous studies, it was stated that as a result of fermentation, free phenolic compounds in the structure of feed increased proportionally and accordingly, antioxidant activity increased (Martins et al., 2011; Tapati and Kuhad, 2014). Similarly, in the current study, it was observed that there were significant differences in DPPH inhibition values of feeds before and after fermentation (Table 2). Tosun (2017) determined that the antioxidant levels of apple and tomato pulps subjected to fermentation with *Aspergillus niger* for 72 hours increased significantly. Bhanja and Kuhad (2014) reported in their solid state fermentation studies that depending on the high enzyme activity in fermented feeds, phenolic compounds in bound form become free and therefore their antioxidant capacity increases.

There was no significant difference in the numbers of Saccharomyces cerevisiae between the pre- and post-fermentation groups. However, at the end of a 48-hour fermentation period, it was determined that the yeast counts of the feeds increased significantly ($P<0.01$) compared to the pre-fermentation period.

Probiotics are alternative feed additives that suppress harmful microorganisms in the intestines of animals, help increase beneficial microorganisms, prevent toxic secretions and strengthen the immune system (Ülger et al., 2015). Yeast culture (*Saccharomyces cerevisiae*), which is a single cell protein source, is an important probiotic feed additive used in poultry rations. Yeast, which is a very good source of protein and amino acids, consists of 75% water and 25% dry matter. Approximately 45-55% of yeast dry matter consists of crude protein. Yeasts, which are very rich in B group vitamins and vitamin E, also contain phosphorus, potassium, magnesium, zinc, chromium, selenium, iron and manganese (Pamir, 1985).

It has been obtained with positive results from the use of yeasts, which are rich in many nutrients, in animal nutrition (Abdelrahman, 2013; Cruz et al., 2020).

The use of industrial wastes such as whey and fruit pulp in yeast production is very important in terms of recycling or converting such wastes into food (Ukaegbu-Obi, 2016). In the aforementioned study, it was observed that there was a significant increase in the yeast count of sunflower seed meal fermented with whey. In other words, with this study, probiotic properties were added to sunflower seed meal.

CONCLUSION

This study was carried out to make sunflower seed meal with pomegranate peel added, which has a high antioxidant rate, more useful for poultry by subjecting it to solid state fermentation with whey.

In the present study, it has been shown that there was no significant change in dry matter and crude fiber ratios in the mixture samples formed by subjecting sunflower seed meal to solid state fermentation with whey, a significant increase in crude ash and crude protein ratios, and a decrease in crude oil ratios. On the other hand, a significant increase in antioxidant capacity and phytase activity, and a decrease in phytic acid level were detected in the mixture samples.

In this study, the highest increase in crude protein ratio was detected in the SM + W + 0.5% PP group, the highest antioxidant activity and the lowest phytic acid ratio in the SM + W + 1.5% PP group. It was concluded that the use of these ratios in poultry feeding would be beneficial. In conclusion, sunflower meal with functional properties was obtained. In addition, wastes such as whey and pomegranate peel were used as feed and environmental pollution was prevented.

CONFLICT OF INTEREST

The authors declare no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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