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Research Article

Effect of Different Ratios of Pomegranate Peels on Hematological, Biochemical Parameters and Reproductive Hormones of Karadi Ram Lambs

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ABSTRACT

The object of current study was conducted to determine the effect of different levels of pomegranate peels (PP) (0, 1, 2or 4%) in the diet of Karadi lambs on Hematological, biochemical and reproductive hormones. Sixteen Karadi male lambs of 4.5-5 months old and weighing 24.29±0.42 kg were randomly divided equally in to four treatment groups and individually penned for a period of 63 days. Results revealed that there were no significant effects of the different levels of pomegranate peel in diets on red blood cell count (RBC), hemoglobin (Hb), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), Neutrophils, Basophils, eosionphils, serum glucose , total protein, enzymes asparttate Aminotranferase (AST), alanine aminotransferase (ALT), serum hormones such as follicle stimulation hormone (FSH), triiodothyronin (T3), thyroxine (T4) and thyrotropin stimulating hormone (TSH). Karadi lambs fed 1% pomegranate peel (PP) had significantly ($P<0.05$) highest value for PCV %, WBC and monocytes, serum hormone luteinized hormone (LH) compared with other treatments. It was concluded that lambs fed 1%PP is their diet showed better immunity and reproductive hormones compared to other treatment groups.

Key words: Blood parameters, Reproduction hormones, Pomegranate peel, Karadi lambs

INTRODUCTION

The pomegranate peels (PP) make up about 60% of the fruit, and they are rich in many compounds such as phenolics, flavonoids, ellagitannins (including punicalagins), proanthocyanidin compounds, complex polysaccharides, and many minerals including potassium, nitrogen, calcium, magnesium, phosphorus, and sodium (Viuda-Martos *et al*., 2010). However, its biological properties are mainly associated with the presence of flavonoids and tannins. The peel is the part of the fruit with the highest antioxidant activity, which is in line with its high content of polyphenols (Guo *et al*., 2003; Li *et al*., 2006). Moreover, pomegranate peels also showed higher antioxidant capacity in vitro when compared to other fruits such as mangos, bananas, and coconuts (Okonogi *et al*., 2007).

Pomegranate peel is a good source of antioxidant and thus may serve in the prevention of cattle diseases and improvement of beef products making it an attractive component in cattle feed. Shabtay *et al.* (2008) demonstrated that dietary supplementation of (PP) promoteed an increase in feed intake, with a tendency to increase weight gain in bull calves. Oliveira *et al.* (2010) found that feeding a pomegranate extract to young calves for the first 70 day of life suppressed intake of grain and whole tract digestibility of fat and crude protein, likely because of its high tannin content. Recent studies also have shown that boosting antioxidant levels in the diet of cattle may help to improve their health. The peel packs some of the weight boosting and health enhancing effects of antibiotics and hormones without the detrimental effects and it may yield meat with higher level of beneficial antioxidants (Shabtay *et al*., 2008). Antioxidants also play an important role in food quality preservation due to their ability to prevent oxidative deterioration of lipids (Erukainure *et al*., 2012).

Blood is useful for assessing the health status, clinical evaluation for survey of physiological/ pathological conditions and diagnostic and prognostic evaluation of various types of diseases in animals (Obasoyo *et al*., 2005 and Amel *et al*., 2006). Hematological analysis involves the determination of different blood parameters such as packed cell volume, red blood cell count among others, which can be done using either the electronic quantification or manual quantification (Wikihow, 2013).

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Zeweil *et al.* (2003) reported that Pomegranate peel had significantly increased ejaculate volume, seminal plasma fructose , improved sperm motility , sperm total output and reduced dead sperm with diets containing 1.5, 3.0 and 4.5% of PP in male rabbits compared to the heat stressed control animals. The aim of this study was to determine the effect of supplementation of pomegranate peel in the diets of Karadi male lambs on hematological, serum biochemical and reproductive hormones.

MATERIALS AND METHODS

Location of experiment

This experiment was carried out at the Animal Farm, Dept. of Animal Production, Faculty of Agric. Sci. Univ. of Sulaimani, Bakrajo, Sulaimani, Kurdistan, Iraq.

Preparation of pomegranate peels

Mature pomegranate fruits were washed and cut manually to separate the seeds and peel. The rind (peels) thus obtained, cut into small pieces using a sharp knife and dried in an air circulatory tray drier at 60±5ºC for 6 h till its moisture content reached 12-14%. Dried pieces were cooled, powdered in a laboratory disc mill to pass through 20 mesh sieve, packed in high density polyethylene bags and stored at ambient temperature (25±5ºC) until use (Singh and Sethi, 2003; Devatkal and Naveena, 2010). The chemical composition namely moisture, crude protein, crude fat, ash, crude fibers and carbohydrates content of pomegranate peels powder are shown in Table 1.

Experiment diets and animals

A total of 16 Karadi male lambs were used with average initial weight of 24.29±0.42 kg and 4-5 months old. All lambs were individually housed and treated against ecto- and endo-parasites. After an adaptation periods of 14 days, lambs were randomly divided equally in to four treatments for a period of 63 days. Four rations were used in this experiment, which were contained one of four levels of Pomegranate peels (PP) (0, 1, 2, or 4%). Chemical composition and formulation ingredients' diets are presented in Table (2).

Lambs of the control group received a basal diet with 0% PP, whereas lambs in T1, T2 and T3 received PP at a rate of 1, 2 or 4%. All lambs were fed concentrate at a rate 3% of their body weight. Twice daily and the refusal of the diet was collected and weighed before offering the feed in the next morning. Barley straw was given *ad libitum*. Clean water was available constantly. Lambs were weighed at weekly intervals.

Chemical analysis of the concentrate diets

In this experiment, concentrate diets were used which contained: barely, yellow corn, soy bean meal, vitamins and minerals mixture. Barley straw was used as a source of roughage *ad libtum*. Samples of feedstuff, offered feed and refusals were dried at 50 ^{o}C until constant weight before chemical analyses. Samples then ground through a 1 mm screen for chemical analysis. Dry matters (DM), organic matter (OM), ether extract (EE), crude fiber (CF), nitrogen free extract (NFE), neutral detergent fiber (NDF) and acid detergent fiber (ADF) were determined according to FAO (2011).

Table 1: Chemical composition of pomegranate peels powder and barley straw

Item %	Pomegranate peels (PP)	Barley Straw
Organic matter (OM)	96.2	91.6
Crude protein (CP)	5.1	4.1
Ether extract (EE)	4.9	1.2
Total Ash	3.8	8.4
Crude fiber (CF)	11.22	41.0
Nitrogen free extract (NFE)	80.5	45.3
Metabolizable energy ME (MJ/Kg)*	27.92	6.6**

*ME was calculated according to Mirzaei-Aghsaghali (2011); **ME of barley straw was calculated according to Hassan *et al*. (2012).

Table 2: Formulation and chemical composition of experimental diets

Ingredients (%)	0%PP	1%PP	2%PP	4%PP
Barley Grain	40	40	40	40
Wheat Bran	28	27	26	24
Yellow Corn	20	20	20	20
Soybean meal	10	10	10	10
Pomegranate Peels	0	1	2	4
Salt(NaCl)	1	1	1	1
Minerals and vitamins	0.5	0.5	0.5	0.5
Dicalcium Phosphate	0.5	0.5	0.5	0.5
Chemical composition%				
Organic matter (OM)	93.7	93.77	93.81	93.87
Crude protein (CP)	15.7	15.6	15.5	15.3
Ether extract (EE)	3.12	3.12	3.13	3.14
Total Ash	6.3	6.23	6.19	6.13
Crude fiber (CF)	7.8	7.7	7.6	7.5
Nitrogen free extract (NFE)	67.08	67.35	67.58	67.93
Metabolizable energy ME (MJ/Kg)*	12.63	12.65	12.67	12.69

*ME (MJ/ kg DM) = 0.012 CP +0.031 EE+0.005 CF +0.014 NFE (MAFF, 1975).

Haematological, biochemical and reproductive hormones parameters

The Karadi lambs were bled through jugular vein and 10ml of blood collected after 6 hrs post feeding at 60 days of the experiment. A sample of 3ml of blood was collected into plastic tube containing EDTA for hematological studies. The remaining 7ml of blood samples were deposited in anti-coagulant free plastic tube and allowed to clot at room temperature within 3hrs of collection then centrifuged at 3000 rpm for 20 min for serum separation. The serum samples were separated at once for determination of biochemical tests. Hematological parameters included red blood cells (RBC), white blood cell (WBC),WBC differentials, mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), mean corpuscular volume (MCV), Packed cell volume (PCV) and hemoglobin concentration values were measured by using hematology analyzer (ACCENT 200, Cormay). Biochemical tests such as blood glucose, serum aspartate aminotransferase(AST), serum alanine aminotransferase (ALT) and alkaline phosphatase(ALP), total protein, albumin and globulin concentration using reagent kits (PZ CORMAY S.A., Poland) with auto biochemistry analyzer (Model accent 200, Poland). Serum hormones analysis such as luteinized hormone (LH), follicle stimulation hormone (FSH), triiodothyronin (T3), thyroxine (T4) and thyrotropin stimulating hormone (TSH) hormone were measured by radioimmunoassay (Model accent 200, Poland).

Statistical analysis

The obtained data were analyzed according to XLSTAT, (2007) as one way analysis of variance according to the following model. Differences among means were carried out by using Duncan's (1955) multiple range tests.

$Yij = \mu + ti + eij$

Where: Yij = the dependent variable, μ = overall mean, Ti = effect of the treatment (i = control, 1%PP, 2% PP or 4% PP), eij = random residual error.

RESULTS

Hematological parameters of Karadi lambs

Table (3) shows the hematological parameters of Karadi lambs fed different level of pomegranate peel (PP) during the experiment period. Karadi lambs fed 1% pomegranate peel (PP) had highest value for PCV %, WBC and monocytes compared with other treatments. The highest mean value (28.89%, 12.39 10^9/ L and 4.0%) for PCV %, WBC and Monocytes were recorded for lambs fed 1%PP while lambs fed 2 or 4 % PP having the least value (26.27%, 11.92 10^9/ L, 3.0% and 26.48%, 10.44 10^9/ L , 3.0%), respectively. But, lambs fed 2 % PP had the lowest value (58.5 %) for lymphocytes compared to control group and lambs fed 1 or 4 % PP (60.9, 60.0 and 59.6 %), respectively. There were significant differences ($P<0.05$) in all the above parameters considered. However, other blood parameters such as RBC, Hb concentration, MCV, MCH, MCHC, Neutrophils, Basophils and Eosinophils were not significantly different ($P>0.05$) among treatment groups.

Biochemical parameters of Karadi lambs

Table (4) shows the observed biochemical parameters of Karadi lambs as affected by the treatments at the end of the experiment. The result of the biochemical parameters (serum glucose, AST, ALT and ALP) showed no significant difference ($P>0.05$) among treatments. However, the mean values (91.0 and 58.9) for serum glucose and alkalinephosphatase(ALP) were highest in lambs fed 4%PP . While, the mean values (38.9, 37.2, 35.0 and 31.1) for AST was highest in lambs fed 2%PP, 1%PP, 4%PP or 0%PP. The mean values of alanine aminotransferase (ALT), was highest in lambs of the control group (10.47) followed by lambs fed 4%PP, 2%PP or 1%PP being 9.23, 8.87 and 7.20, respectively.

Reproductive hormones in Karadi lambs

Serum reproductive hormones of Karadi lambs fed pomegranate peel are shown in Table (5). Follicle stimulation hormone (FSH), triiodothyronin (T3), thyroxine (T4), thyrotropin stimulating hormone (TSH) did not significantly differ ($P>0.05$) among treatments. But, the mean value (1.663 ng/ ml) for luteinized hormone (LH) was highest in lambs fed 1%PP as compared to the lower value (1.208) in Karadi lambs fed control diet. There were significant differences ($P<0.05$) in all treatment groups for luteinized hormone (LH) in Karadi lambs.

Table 3: Effect of different levels of pomegranate peels on hematological parameters of Karadi lambs (Mean ± SE)

Parameter	0%PP	1%PP	2%PP	4%PP
RBC (10^{12}/ L)	13.83±0.38^a	13.90±0.15^a	13.31±0.35^a	13.23±0.25^a
Hb (g/L)	84.83±1.81^a	84.93±1.38^a	85.50±1.82^a	84.82±1.29^a
PCV%	27.13±0.55^a	28.89±0.14^a	26.27±0.19^b	26.48±0.54^b
MCV(fl)	29.62±0.21^a	29.08±0.52^a	29.77±0.67^a	29.03±0.40^a
MCH (Pg)	8.14±0.26^a	8.31 ±0.22^a	8.19 ±0.46^a	8.37±0.46^a
MCHC (g/dl)	31.46±0.16^a	31.75 ±1.20^a	32.59±1.02^a	32.44±1.23^a
WBC(10^9/ L)	10.11±0.19^b	12.39±0.26^a	11.92±0.13^a	10.44±0.10^b
Lymphocytes %	52.9±8.63^a	53.0±7.40^a	50.5±8.42^b	50.6±8.26^b
Neutrophils %	42.2±3.41^a	41.0±2.72^a	43.8±2.57^a	43.6±2.25^a
Monocytes %	2.0±0.57^c	4.0±0.57^a	3.0±0.57^b	3.0±0.57^b
Basophils %	0.0±0.00^a	0.0±0.00^a	0.0±0.00^a	1.0±0.57^a
Eosinophils %	2.5±0.25^a	2.0±0.57^a	2.7±0.23^a	2.8±0.26^a

a, b, c : means on the same row with different superscripts are significantly different ($P<0.05$); RBC: red blood cell, Hb: Hemoglobin, PCV: Packed cell volume, MCH: mean corpuscular hemoglobin, MCHC: mean corpuscular hemoglobin concentration, WBC: white blood cell

Table 4: Effect of different levels of pomegranate peels on serum biochemical parameters of Karadi lambs (Mean ± SE)

Parameter	0%PP	1%PP	2%PP	4%PP
Serum glucose (mg/dl)	77.05±5.79^a	81.54±2.10^a	80.08±4.74^a	81.0±4.20^a
AST (U/L)	37.1±1.98^a	37.2±3.53^a	38.9±5.55^a	36.9±3.18^a
ALT(U/L)	10.47±0.62^a	8.20±0.90^a	8.87±1.10^a	9.23±1.17^a
ALP (U/L)	78.0±8.52^a	73.8±10.94^a	74.5±8.01^a	78.9 ±10.39^a

a, b, c : means on the same row with different superscripts are significantly different ($P<0.05$); AST : aspartate amino transferase, ALT: alanine aminotransferase, ALP: alkaline phosphatase

Table 5: Effect of different levels of pomegranate peels on reproductive hormones of Karadi lambs (Mean ± SE)

Hormones	0%PP	1%PP	2%PP	4%PP
LH(ng/ ml)	1.208±0.051^c	1.663±0.090^a	1.393±0.094^{bc}	1.470±0.061^{ab}
FSH(ng/ ml)	0.359±0.276^a	0.350±0.230^a	0.130±0.015^a	0.127±0.015^a
T3 (n.Mol/L)	3.357±0.419^a	3.093±0.549^a	4.443±0.708^a	3.217±0.651^a
T4 (n.Mol/L)	99.73±6.010^a	105.80±8.598^a	123.00±9.167^a	142.400±8.182^a
TSH(u IU/ml)	0. 24 ±0.04^a	0. 27 ±0.08^a	0. 28±0.01^a	0. 26±0.02^a

a, b, c : means on the same row with different superscripts are significantly different ($P<0.05$); LH: luteinized hormone, FSH: follicle stimulation hormone, T3: triiodothyronin, T4: thyroxine ,TSH: thyrotropin stimulating hormone.

DISCUSSION

In the study, the results showed that different levels of pomegranate peel (PP) has no effects on all hematological parameters in Karadi male lambs. The experimental animals, particularly those consuming 1 % PP diets did not show clinical signs of ill health or signs of tannin toxicity such as brisket oedema, diarrhoea, constipation, anorexia, hard pelleted feces coated with blood and mucous (Garg *et al*., 1992) because of that pomegranate peel which contained tannin. The absence of signs of ill health and mortality in the animals consuming PP in the diet of this experiment, in which tannins were more concentrated, confirms the non-toxic level of tannins in PP. The high significant in PCV for lambs fed on 1 % PP diets could be attributed to the presence of anti nutritional factors (Table 3), particularly phenols and condensed tannins, that have been reported to have an antinutritional action (Robins and Brooker, 2005; Rubanza *et al*., 2005). However, these values were within the ranges of 22-38% for PCV, reported for clinically healthy sheep (Merck, 2012). So, in this study, lambs fed 2 or 4% had lower significant value of PCV compared to control or 1% PP. Parallel results were obtained by Mahgoub *et al*. (2008), who observed low PCV for sheep fed non-conventional feeds containing condensed tannins and phenols. The normal RBC values elucidated the absence of haemolytic anaemia and depression of erythrogenesis. All lambs have not significantly different for hemoglobin concentrations and RBC were within the reported range (80-160 g/L) and (8-15×10^{12}/ L) for lambs (Coles, 1986) suggesting the absence of microcytic hypochromic anaemia occasioned by iron deficiency and improper utilization for the formation of Hb. The fact that the blood indices (MCV, MCH and MCHC), which are important for diagnosis of anaemia in most animals (Coles, 1986), were within the normal ranges (16-25 fL, 5-8 fmol and 28-34 g/dl), respectively (Table 3), Merck (2012) showed explicitly that the sheep were not anaemic. So, in this result lambs fed on pomegranate peel (PP), they have not occurred anaemia disease. The lack of treatment effect on MCV, MCH and MCHC values agreed with earlier reports (Solaiman *et al*., 2010). Oak (*Quercus incana*) poisoning resulting in reduced Hb, MCH, increased bilirubin above the normal ranges and 70% mortality observed in cattle consuming tannin-rich immature tender oak leaves (Garg *et al*., 1992). The lower WBC, lymphocyte and monocyte counts for the lambs fed 4% PP in the diet may be connected with the tannins concentration of this diet. May be the tannin content of this diet would be higher than in the other diets, which implies that the animals must have ingested a considerable amount of tannins. Though the animals had lower WBC, lymphocyte and monocyte counts, the values were within the ranges (4-12 × 10^{9} L), 40-55% and 0-6%), respectively, reported for healthy sheep (Jain, 1993 and Merck, 2012). This could be an important indication of the health status of the experimental animals and confirms the earlier conjecture that the concentration of tannin in this diet was lower than the level that could have induced toxicity or ill health in the animals. Karadi lambs fed 1% PP had highest value for lymphocytes and monocytes compared with other treatments. 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*., 2008). Generally, toxic substances in feed 4% PP tend to suppress haemopoietic tissues with consequent production of a low WBC count. The results agreed with that of Mahgoub *et al*. (2008) who also indicated low lymphocyte and monocyte counts in sheep fed tannin-rich non-conventional feeds.

Serum glucose in this study was not significantly among treatment may be diets similar energy content of the four diets (Table 4). Since the levels were within the normal range (44-81 mg/dl) indicated for healthy sheep (Kramer, 2000). Serum levels of AST, ALP are those conventionally used for diagnosing human and domestic animal hepatic damage (Silanikove and Tiomkin, 1992). Specifically, ALP is used to detect bile obstruction, i.e. mild and progressive damage to the liver (Silanikove and Tiomkin, 1992), whereas liver enzymes like ALT, which is a liver specific hepatocellular enzyme released by hepatocellular damage, is used to assess liver damage (Mahgoub *et al*., 2008). All enzymes in this study with in the normal ranges for ALT, AST and ALP are 5-21.6 IU/L, 36-115 IU/L and 73-217 IU/L in Awassi male lambs at 7- 9 month (Al-Hadithy *et al*., 2013).

Higher significant reproductive hormone like Luteinized hormone (LH) in male lambs fed 1% PP compared to other treatment(Table5) may be has appositive effect of pomegranate peel for enhance sexual hormones and more digestible PP by lambs because of pomegranate peel has contained vitamin E and it is affected of accumulate of vitamin E in blood circulation during feeding trial in Karadi male lambs, it is similar results obtained by (Shabtay *et al*., 2008) who reported that increased plasma α-Tocopherol concentration in pomegranate peel consuming by calves along the feeding experiment for 8 weeks . The fact that none of these blood metabolic profiles in the present study differed from within the normal ranges for lambs suggest that no damage to the liver occurred. The result, which is in agreement with that of Silanikove *et al*. (1996), but contradicts the report that the antinutritional factors in Prosopis julifloral pod diets caused tissue damage in goats (Tabosa *et al*., 2000).

Conclusion

From the present study, it can be concluded that the hematological and biochemical parameters for Karadi lambs fed different levels of PP fall within normal range. The absence of clinical signs of ill health, tannin toxicity symptoms and the findings of all the hematological and serum metabolites within the established ranges for healthy Karadi lambs suggest that 1% pomegranate peel in the diet were well tolerated by the Karadi lambs. It was thus concluded that the supplementation of 1% pomegranate peel did not cause any major health disorders and improve immunity and LH reproductive hormone in Karadi male lambs.

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