Short Paper

The effect of long-term feeding of olive-pulp silage on blood attributes of two fat-tailed ram breeds

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(Received 23 Feb 2013; revised version 20 Oct 2013; accepted 27 Oct 2013)

Summary

The effect feeding olive-pulp silage (OPS) for 120 days on several blood attributes was studied using 16 rams allotted to two diets consisting of 70% corn silage or 70% OPS. Olive-pulp silage feeding resulted in lower serum total protein, alanine aminotransferase, calcium and phosphorus levels and higher glucose concentration compared to the corn silage diet. Body weight and other blood attributes were not affected by OPS feeding. Feeding a diet containing 70% OPS at the maintenance level had no apparent detrimental effect in mature rams.

Key words: Blood biochemistry, Hematology, Olive pulp, Ram, Sheep

Introduction

Non-conventional feedstuff such as olive by-products have been used as animal feed (Molina-Alcaide and Yanez-Ruiz, 2008; Taheri *et al*., 2012) but their anti-nutritional compounds (Makkar, 2003) may increase protein output in faeces (Robins and Brooker, 2005) and negatively affect animal performance (Mahgoub *et al*., 2008) and health (Walton *et al*., 2001). Despite publication of several studies on the nutritional effects of short-term feeding of olive-oil by-products in sheep (Hadjipanayiotou, 1994; Al Jassim *et al*., 1997; Ben Salem *et al*., 2002), longer term feeding trials, especially in breeding animals, are scarce. In most experiments, olive by-products did not constitute a large portion of the diet (Mioc *et al*., 2007; Yanez-Ruiz and Molina-Alcaide, 2007), which may have masked its harmful effect(s). This experiment was conducted to determine the effect of feeding a diet containing 70% OPS for 120 days on changes in blood attributes in mature rams.

Materials and Methods

After three weeks of adaptation, 16 (3.5 to 4.0 years of age; 53 to 78 kg live weight) Ghezel or Mehraban rams (8 rams per breed) were fed with maintenance diets (NRC, 2007) containing 30% alfalfa hay with 70% olive-pulp or corn silages for 120 days (Table 1), having free access to fresh water and mineral blocks. Jugular blood samples were taken on days 0, 60, 120, and 30 days after the animals were fed with the pre-experimental diet. The general husbandry practices were described earlier (Poornahavandi and Zamiri, 2008).

Table 1: Ingredients and composition of the diets (Dry matter basis)*

Ingredients and composition	Olive-pulp silage	Corn silage
Ingredient (g/kg)		
Olive-pulp silage (CP=120)	700	-
Corn silage (CP=114)	-	700
Alfalfa hay (CP=120)	300	300
Composition		
Metabolizable energy (MJ/kg)	9.62	10.13
Crude protein (g/kg)	120.0	116.0
Ether extract (g/kg)	117.3	24.6
Neutral detergent fiber (g/kg)	519.7	593.8
Ash (g/kg)	58.7	63.1
Calcium (g/kg)	7.6	6.6
Phosphorus (g/kg)	1.8	2.0

* On a daily basis, rams received either 1.5 kg olive-pulp silage (70% DM) and 0.7 kg alfalfa hay (90% DM) or 4.4 kg corn silage (30% DM) and 0.7 kg alfalfa hay

Whole blood samples were used for total red blood cell count (RBC), hematocrit (at 11000 rpm; 7 min), total white blood cell count (WBC; by hemocytometer), and differential WBC count (giemsa staining). Serum sample was prepared (3000 rpm; 15 min) and stored at -20°C until analysed (Thomas, 1998) for glucose (glucose oxidase-phenol aminophenazone method), blood urea nitrogen (BUN; UV kinetic method), albumin (bromocresol green method), creatinine (Jaffe method), total protein (biuret method), alanine aminotransferase (ALT; international federation of clinical chemistry or IFCC reference method) alkaline phosphatase (ALP; deutschen gesellschaft fur klinische chemie or DGKC kinetic method), calcium (Arsenazo III), phosphorus (UV method) and iron (ferene method) levels, by spectrophotometric methods using Cobas Mira

Chemistry Analyzer (Roche, Germany).

Data were analysed using the PROC MIXED (SAS, 2002) for repeated measure data. Body weight was included as a covariate, and means were compared by the Tukey's test (P≤0.05).

Results

Live weight remained unchanged throughout the trial (Table 2). Interaction effects were not significant. Except for small differences in the percentages of neutrophils, monocytes and eosinophils, phosphorus level, other measurements were not affected by breed. Feeding OPS decreased serum total protein, ALT, calcium, and phosphorus, but increased glucose; other blood attributes were not affected by the diet (Table 3). Time of sampling significantly influenced the values for hematocrit, RBC count, BUN, creatinine, ALT, phosphorus and iron (Table 4). The highest hematocrit value was recorded on days 0 and 150. Red blood cell count was lowest on day 120. Serum levels of BUN, ALT and phosphorus were highest but iron level was lowest on day 150.

Discussion

Non-significant changes in live weight during the experiment indicated that OPS-diet, formulated to satisfy the maintenance requirements, was adequately consumed, without apparent side effects, at least in terms of live weight and duration of the trial.

Red blood cell count and hematocrit were lowest on day 120 (in July) and lower than the reference normal physiological values (Coles, 1986). Dehydration can result in high hematocrit values (Swenson, 1977); however, the rams could not have suffered from dehydration as they had free access to water. Feeding OPS decreased serum total proteins; nevertheless, the values were within the normal physiological ranges (Coles, 1986). This might be due to the lower degradability of protein in olive by-products (Theriez and Boule, 1970; Molina-Alcaide and Yanez-Ruiz, 2008) that can be attributed to high ADF content of low degradability, which, in turn, decreases protein degradability; 75 to 90% of nitrogen in olive by-products is bound to ADF (Nefzaoui, 1978). Increased efficiency

Table 2: Live weight (kg) of rams as affected by the diet

Silage	Days				
	0	60	120	150	Pooled SE
Olive pulp	64.7	66.1	64.7	65.6	4.83
Corn	64	64.4	65.8	66.0	5.72

Table 3: Least squares means of blood attributes in rams fed olive-pulp or corn silages

Blood attributes	Olive-pulp silage	Corn silage	Pooled SE
Hematocrit (%)	34.3	33.4	0.95
Red blood cells ($\times 10^6/\mu L$)	8.20	8.26	0.21
White blood cells ($\times 10^3/\mu L$)	5.12	4.69	0.25
Neutrophils (%)	53.2	60.4	3.50
Lymphocytes (%)	43.4	36.2	3.42
Monocytes (%)	1.2	1.3	0.28
Eosinophils (%)	1.8	2.1	0.36
Glucose (mg/dL)	67.9*	61.4	2.10
Blood urea nitrogen (mg/dL)	16.1	15.4	1.09
Creatinine (mg/dL)	1.27	1.35	0.08
Globulin (g/dL)	3.9	4.3	0.28
Albumin (g/dL)	3.8	3.7	0.14
Total protein (g/dL)	7.7*	8.2	0.12
Alanine aminotransferase (U/L)	17.2*	21.1	1.27
Alkaline phosphatase (U/L)	389	391	57.2
Ca (mg/dL)	9.8*	10.2	0.14
P (mg/dL)	6.3*	7.4	0.23
Fe (µg/dL)	200	196	15.90

* Significantly different from corn silage (P<0.05)

Table 4: Effect of sampling time on selected blood attributes in rams (least squares mean)

Blood attributes	Time (day)				
	0	60	120	150	Pooled SE
Hematocrit (%)	35.6^{a}	33.4^{b}	31.9^{b}	34.2^{a}	1.48
Red blood cells ($\times 10^6/\mu L$)	8.5^{a}	8.2^{a}	7.3^{b}	8.9^{a}	0.40
Blood urea nitrogen (mg/dL)	13.4^{b}	13.6^{b}	12.1^{b}	24.0^{a}	1.55
Creatinine (mg/dL)	1.4^{ab}	1.3^{bc}	1.3^{bc}	1.2^{cd}	0.10
Alanine aminotransferase (U/L)	16.5^{b}	18.1^{b}	18.9^{b}	23.2^{a}	2.02
P (mg/dL)	6.4^{b}	6.4^{b}	7.2^{a}	7.3^{a}	0.45
Fe (µg/dL)	226^{a}	209^{a}	191^{ab}	167^{b}	23.81

[a, b] Within row, means with common superscript(s) are not different (P>0.05). Other attributes were not affected by time

of microbial protein synthesis and decreased ruminal protein degradability resulting in increased supply of non-ammonia nitrogen to the small intestine following feeding of tannin-rich feeds (Makkar, 2003) were not confirmed in our study, where lower serum levels of total protein were recorded in OPS-fed rams. Decreased availability of precursors for liver synthesis of proteins would have been more probable than the lower functionality of hepatocytes, as serum ALT level did not increase in OPS-fed rams.

Feeding OPS decreased serum levels of calcium and phosphorus, with phosphorus values being within the normal physiological ranges, and serum calcium levels lower than the published ranges (Coles, 1986). These could be due to lower digestibility and/or an imbalanced mineral intake, as was seen in sheep feeding diets containing phenols and condensed tannins (Mahgoub *et al*., 2008). Hypoproteinemia may lower serum calcium, as protein-bound calcium constitutes up to 50% of total plasma calcium (Coles, 1986).

Serum ALP was not affected by OPS but Yanez-Ruiz and Molina-Alcaide (2007) reported an increase in ALP level in wethers and goats fed olive leaves *ad libitum* for 28 days. This discrepancy might be due to factors such as the proportion of different physical components (skin, pulp and water), residual oil extraction, year, geographic origin, and contamination with soil (Molina-Alcaide and Yanez-Ruiz, 2008) and probable deficiencies in zinc and vitamin B6 content. The highest level of ALT on day 150 could not be a consequence of treatment, since OPS feeding was terminated one month earlier.

Unlike the study by Mahgoub *et al*. (2008), serum glucose levels increased in OPS rams, indicating that OPS was not a feed of inferior nutritional value in this respect, as also supported by live weight data. Increased glucose levels might be due to high oil contents in olive-pulp (Molina-Alcaide and Yanez-Ruiz, 2008), thus sparing blood glucose; however, OPS oil content was not measured in our study. Creatinine decreased as the duration of OPS feeding increased; but, the precise cause of this decrease is not known, as body weight remained almost constant during the experiment.

The current data, and those indicating no adverse effect on seminal attributes (Faraji *et al*., 2012), show that a diet containing 70% OPS and 30% alfalfa hay is unlikely to have a major detrimental effect in mature rams when fed up to 120 days. As a cheap by-product, olive-pulp may used as an economical feed for mature rams during extended periods; however, it remains to be determined if OPS feeding can cause subtle histological changes in the body.

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