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Original Research Article

Influence of dietary hydrogenated palm oil supplementation on serum biochemistry and progesterone levels in dairy goats

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ABSTRACT

The aim of this research was to investigate the influence of hydrogenated palm oil (HPO) added to a dairy goat diet on serum biochemistry and progesterone levels. Thirty pregnant Cilentana dairy goats were equally divided into 2 groups (control [CTR] and HPO groups). After kidding, concentrated feed for both groups was gradually increased up to 400 g/(animal·d), and the HPO group received 50 g/(animal·d) of HPO. Supplementation with HPO significantly increased cholesterol levels (mg/dL, 63.80 vs. 54.68 at 30 d, $P \leq 0.05$; 78.20 vs. 58.00 at 60 d, $P \leq 0.05$; 83.80 vs. 57.83 at 120 d, $P \leq 0.01$) compared with the CTR group although no significant differences were detected for liver and kidney function indicators. Moreover, other biochemical parameters were not affected by HPO supplementation thus suggesting no change occurred in lipid and protein metabolism. Furthermore, a significant correlation was found between progesterone levels and serum cholesterol ($r = 0.65$, $P \leq 0.01$) although these were not significantly higher in HPO supplemented goats. The dose and time of HPO supplementation appears critical as regards assessing the limits between the risks and benefits of HPO supplementation in dairy goats. At the tested dose, HPO was well tolerated by the animals and may represent a useful tool to increase energy availability during highly demanding periods.

1. Introduction

Nutrition plays a pivotal role in determining metabolic status, and thus influences livestock performance, animal welfare and product quality. More particularly, several studies have demonstrated that the animal's diet greatly influences milk polyunsaturated fatty acid (PUFA) and conjugated linoleic acid (CLA) (D'Urso et al., 2008; Tudisco et al., 2010, 2012, 2014).

The addition of lipids as a source of energy in the diet of dairy animals is aimed at supporting high milk production obtained by genetic improvement of livestock (Oliveira et al., 2012). A high level of milk production may induce a negative energy balance that can be dangerous for animal health, and may result in productive and reproductive losses. Results concerning palm oil supplementation to the diet of ruminants are often controversial. Kupczynski et al. (2012) found no change in milk yield, fat and protein in Holstein-Friesian cows, whereas Duarte et al. (2005) found higher milk production in Jersey cows fed diets with palm oil. In addition, higher levels of short and medium chain fatty acids, as well as C14:0, cis-9 C16:1 and cis-9 trans-11 CLA were found by Tudisco et al. (2015) in milk from goats fed diets with hydrogenated palm oil (HPO).

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Data concerning effects on metabolism are also not superimposable but are often analysed during different productive periods. Agazzi et al. (2010) found no difference in plasma metabolites, nor on liver enzymes in transition goats fed a diet with HPO compared to the control group except for a significant lower plasma alanine-aminotransferase (ALT), although a significant non esterified fatty acids (NEFA) increase was found. Conversely, Bianchi et al. (2014) found increases in serum aspartate-aminotransferase (AST) and γ-glutamyl transpeptidase (GGT) activities in dairy sheep fed a diet containing 6% palm oil for 120 d. Additionally, Bianchi et al. (2014) showed increases in urea, cholesterol (CHO), triglycerides (TRI) and progesterone levels in animals supplemented even with a lower dosage (4%) of palm oil (administered for 60 and 120 d). The effects of HPO supplement have already been investigated. Indeed, those experiments were performed on sheep from the onset of lactation (Bianchi et al., 2014) and goats only during the transition period (Agazzi et al., 2010). In order to obtain a complete picture regarding the possible benefits of HPO in goat, the metabolic, productive and endocrine effects of HPO administration in goats diet was investigated throughout lactation, and specific functional liver and kidney parameters as well as serum lipids and progesterone levels were assayed.

2. Materials and methods

2.1. Animals, diets, and management

All procedures followed Animal Welfare and Good Clinical Practice (Directive, 2010/63/EU) and were approved by the local Bioethics Committee in Italy. The experiment was performed in a farm located at Casaletto Spartano, Salerno province, in southern Italy at 832 m above sea level. Thirty pregnant Cilentana dairy goats (third-parity; 53.0 ± 1.6 kg BW) were equally divided into 2 groups (control [CTR] and HPO groups) homogeneous for previous milk yield (1,480 ± 116 g/[animal·d]). Animals were fed *ad libitum* oat hay and 100, 200, and 300 g/(animal·d) of concentrate starting from 45 up to 30 d, 30 up to 15 d before kidding, and 15 d before kidding up to kidding, respectively. Oat hay was replaced by alfalfa hay (1.2 kg/[animal·d]; CP: 164 g/kg DM; NDF: 442 g/kg DM; NE: 5.34 MJ/kg DM) after kidding, and concentrate was gradually increased up to 400 g/(animal·d). Hydrogenated palm oil group received 50 g/(animal·d) of HPO (Mazzoleni, Prodotti Zootecnici, Cologno al Serio, Bergamo, Italy). Feed chemical composition (Van Soest et al., 1991; AOAC, 2005) and the nutritive values (INRA, 1978) are reported in Table 1. Hydrogenated palm oil fatty acid profile was analysed by means of gas chromatography according to Castro et al. (2009). Fatty acid profile (% of total FA) of HPO was as follows: C12:0, 0.7%; C14:0, 1.3%; C16:0, 48.0%; C18:0, 44.3%; C18:1, 5.1%; C18:2, ω-6, 0.2% and C18:3, ω-3 ≤ 0.10%.

2.2. Milk analysis

Daily milk yield was registered during the trial, while 4 representative milk samples (d 0, 30, 60 and 120) were analysed for protein and fat concentration using a Milkoscan 133B (Foss 6 Matic, Hillerod, Denmark).

2.3. Blood sampling

Blood samples were collected from goats via jugular venipucture before the feeding administration. Individual blood samples were collected into 5-mL Lithium Heparin tubes and 2-mL K_2EDTA tubes and transported to the laboratory within 2 h. Starting at the onset of palm oil supplementation (1 wk after kidding), blood was collected at d 30, 60 and 120. Serum was obtained by centrifugation at 386 × *g* for 15 min, then samples were frozen in small aliquots at − 80 °C.

2.4. Blood chemistry

Blood chemistry analyses were performed by an automatic biochemical analyser (Olympus AU 400, Beckman Coulter-California-USA) using reagents from the same factory to determine blood urea nitrogen (BUN), creatinine (CREA), AST, ALT, CHO and TRI; reagents from Catachem (Bridgeport-Connecticut-USA) to determine beta-hydroxybutyric acid (BHBA); and reagents from Randox (Ireland) to determine NEFA. Progesterone (PG) level was assessed by Enzyme Immunoassay (EIA), according to the manufacturer's instructions (Cayman Chemical Company, Ann Arbor, MI, USA). Results of PG level were expressed in ng/mL.

2.5. Statistical analysis

Results were subjected to the analysis of variance (PROC GLM, SAS, 2000) according to the following model:

$$y_{ijk} = \mu + T_i + S_j + \varepsilon_{ijk}$$

where y = single datum, μ = general mean, T = effect of the dietary treatment (i = CTR and HPO), S = effect of sampling (j = d 0, 30, 60 and 120) and ε = residual error.

Tukey's test was adopted as a multiple-comparison test to determine the source of variation.

The relationship between CHO and PG levels was evaluated by the linear correlation analysis (PROC CORR, SAS, 2000).

3. Results

No differences were detected for goats' BW, and residuals were not found. Milk yield was significantly higher in the HPO group (1,740 vs. 1,580 g of milk/[animal·d]; $P \leq 0.05$). Milk fat was significantly ($P \leq 0.05$) higher in the HPO group (4.14% vs. 3.55%), and no difference was found for protein content (Table 2). In any event, the goats' energy requirements were satisfied. Rubino (1996) reported that, for local genotype goats, the energy requirements for maintenance is equal to 0.0365 UFL/kg $BW^{0.75}$ (1 UFL = 1.7 Mcal) and for milk production is equal to 0.41 UFL/kg 4% fat-corrected milk. In this trial, the animals weighed on average 53.2 kg and ingested 1.2 kg DM of alfalfa hay counting 0.9 UFL (Table 1), and energy requirements were equal to 1.29 and 1.42 UFL for CTR and HPO groups, respectively (0.69 UFL maintenance plus 0.60 and 0.73 UFL/kg milk production, respectively for CTR and HPO groups). The

Table 1
Feed chemical composition and nutritive values.

Item	Crude protein, g/kg DM	Ether extract, g/kg DM	NDF, g/kg DM	ADF, g/kg DM	Lignin, g/kg DM	Energy requirements for maintenance, UFL/kg DM[2]
Alfalfa hay	164.0	15.3	442.3	320.4	50.3	0.75
Concentrate[1]	180.0	30.0	270.0	115.0	30.0	1.03

[1] Ingredients (g/kg DM): soft wheat bran, 300; soybean solvent extracted, 130; corn meal, 130; sunflower meal, 105; citrus pulp, 80; sugar beet pulp, 79; corn gluten feed, 70; sugarcane molasses, 75; $CaCO_3$, 15; $CaHPO_4$, 7; vitamins, 2; NaCl, 7.
[2] UFL: a feed unit for milk production; 1 UFL = 1.7 Mcal.

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Table 2
Milk yield and milk characteristics in dairy goats: Evaluation of diet effect.

Item	Yield, g	Fat, %	Protein, %
CTR group[1]	1.580[b]	3.55[b]	2.98
HPO group[1]	1.740[a]	4.14[a]	3.01
SEM	174.56	0.298	0.069

CTR = control; HPO = hydrogenated palm oil.
[a, b] Within a row, means with different small letter superscripts differ at $P \leq 0.05$.
[1] CTR group: dairy goats were fed a total mixed diet; HPO group: dairy goats were fed the total mixed diet supplemented with HPO at 50 g/(animal·d).

deficit of 0.39 (CTR) and 0.52 (HPO) UFL was made up with concentrate (0.4 kg counts 0.412 UFL) and only in the case of the HPO group also by the energy of fat added.

Hydrogenated palm oil fatty acid profile was characterized by high levels of palm (C16:0, 48.0% of total fatty acid) and stearic (C18:0, 44.3% of total fatty acid) acids.

As depicted in Tables 3 and 4, significantly higher values ($P \leq 0.05$ at the second and third sampling; $P \leq 0.01$ at the fourth sampling) were seen for CHO level in the HPO group compared to the CTR group.

Moreover, a significant time dependent increase of CHO was also recorded in the HPO group. A moderate, not significant increase was seen for PG level in the treated group after 120 d of HPO supplementation. No difference was detected for all other parameters. Cholesterol and PG were significantly correlated in both groups, but mainly in the HPO group ($P \leq 0.05$ and $P \leq 0.01$ for CTR and HPO groups, respectively). Moreover, the positive relation between CHO and PG in the HPO group was also detectable after 30 and 60 d ($P \leq 0.05$) and increased up to 120 d ($P \leq 0.01$).

4. Discussion

In this trial, the influence of the HPO supplementation on serum biochemical profile and progesterone levels in dairy goats was investigated. Administering HPO significantly increased milk yield in goats (Agazzi et al., 2010 and Tudisco et al., 2015), sheep (Castro et al., 2009), and dairy cows (Salado et al., 2004). Also Bianchi et al. (2014) found an increase in milk yield by adding 2%, 4% and 6% of palm oil to the diet of lactating sheep even if the differences were not significant. In addition, the significant higher level of fat in milk of the HPO group is in agreement with the results obtained by Bianchi et al. (2018a, 2018b) in ewes fed a diet supplemented with 4% and 6% of HPO. Hydrogenated palm oil (6% of the diet) increased CHO levels in ewes, but did not influence serum biochemistry profile, according to the results obtained by Bianchi et al. (2018b), thus suggesting that the dose of supplemented HPO was safe for goats in terms of liver and kidney function. Serum CHO increases when ruminants are fed diets with protected fat because of a higher absorption of long chain fatty acids (Nestel et al., 1978). The increase of CHO we found in the goats fed diets supplemented with HPO is due to the high concentration of C16:0 (Ulbricht and Southgate, 1991), and it is in agreement with the results of Beynen et al. (2000) who reported that plasma lipid concentrations in goats respond to the amount and type of fat in the diet. Ghoreishi et al. (2007) found similar results with higher values of PG and TRI in plasma from sheep fed diets with different concentrations of protected fat. Bianchi et al. (2014) found increases of serum AST and GGT in sheep supplemented HPO at 120 d and of BUN, CHO, TRI, and PG levels at 60 d. As a result, the last-mentioned authors concluded that HPO in the diet influences lipid and protein metabolism and causes an increase in liver enzymes related to its administration. This is important since the high doses of lipid in the diet could increase as a consequence of hepatic steatosis due to certain disorders related to energy metabolism damaging hepatocytes (Pechová et al., 2006). Therefore, long-term administration of HPO may impair liver health. Bianchi et al. (2014) found such effects in dairy sheep fed diets with HPO (6% of the diet), but on the contrary, the same authors suggested that 4% might be a safe dose to be used in the diet for 120 d.

In this experiment, the dose of 50 g/(animal·d) of HPO corresponding to 3.0% of the diet could represent an adequate dose to improve production with no health risk for animals. Such a hypothesis is also confirmed by the absence of differences in BUN and CREA levels. Serum BUN is a known marker of protein metabolism, and is, therefore, related to energy:protein ratio of the diet

Table 3
Biochemistry parameters in dairy goats: Evaluation of diet effect.

Item	Group[1]	Level	SEM
AST, U/L	CTR	72.33	8.44
	HPO	70.92	
ALT, U/L	CTR	17.29	4.55
	HPO	19.54	
CHO, mg/dL	CTR	50.00[B]	8.15
	HPO	70.16[A]	
TRI, mg/dL	CTR	19.33	2.79
	HPO	20.12	
NEFA, mmol/L	CTR	0.362	0.13
	HPO	0.358	
BHBA, mmol/L	CTR	0.369	0.10
	HPO	0.339	
BUN, mg/dL	CTR	39.75	5.65
	HPO	38.96	
CREA, mg/dL	CTR	0.950	0.30
	HPO	1.030	
PG, ng/mL	CTR	2.690	0.70
	HPO	2.890	

AST = aspartate-aminotransferase; ALT = alanine-aminotransferase; CHO = cholesterol; TRI = triglycerides; NEFA = non esterified fatty acids; BHBA = beta-hydroxybutyric acid; BUN = blood urea nitrogen; CREA = creatinine; PG = progesterone; CTR = control; HPO = hydrogenated palm oil.
[A, B] Within a row, means with different capital letter superscripts differ at $P \leq 0.01$.
[1] CTR group: dairy goats were fed a total mixed diet; HPO group: dairy goats were fed the total mixed diet supplemented with HPO at 50 g/(animal·d).

Table 4
Biochemistry parameters in dairy goats: Evaluation of time effect.

Parameter	Group[1]	Time, d				SEM
		0	30	60	120	
AST, U/L	CTR	74.33	73.83	70.83	70.33	8.44
	HPO	80.40	68.40	68.20	67.40	
ALT, U/L	CTR	18.00	17.50	16.33	17.33	4.55
	HPO	17.20	18.80	18.80	20.20	
CHO, mg/dL	CTR	57.50	54.67[b]	58.00[b]	57.83[B]	8.15
	HPO	56.20	63.80[a]	78.20[a]	83.80[A]	
TRI, mg/dL	CTR	19.00	19.67	19.33	19.33	2.79
	HPO	21.00	20.80	20.20	19.80	
NEFA, mmol/L	CTR	0.36	0.36	0.36	0.37	0.13
	HPO	0.34	0.39	0.35	0.40	
BHBA, mmol/L	CTR	0.34	0.38	0.37	0.39	0.10
	HPO	0.43	0.33	0.33	0.32	
BUN, mg/dL	CTR	40.17	39.83	38.33	40.67	5.65
	HPO	42.20	41.40	37.00	39.00	
CREA, mg/dL	CTR	1.00	0.98	1.18	0.83	0.30
	HPO	1.06	0.93	1.03	0.86	
PG, ng/mL	CTR	2.50	2.80	2.22	3.25	0.70
	HPO	2.44	3.06	3.02	3.34	

AST = aspartate-aminotransferase; ALT = alanine-aminotransferase; CHO = cholesterol; TRI = triglycerides; NEFA = non esterified fatty acids; BHBA = beta-hydroxybutyric acid; BUN = blood urea nitrogen; CREA = creatinine; PG = progesterone; CTR = control; HPO = hydrogenated palm oil.
[a,b,A,B] Within a row, means with different small letter superscripts differ at $P \leq 0.05$, and with different capital letter superscripts differ at $P \leq 0.01$.
[1] CTR group: dairy goats were fed a total mixed diet; HPO group: dairy goats were fed the total mixed diet supplemented with HPO at 50 g/(animal·d).

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(Wittwer, 2000). In our study, HPO did not activate the metabolism of proteins as hypothesized by Bianchi et al. (2014) who attributed their results to the different content of each ingredient in the diets thus hypothesizing an unknown mechanism for fats administration linked to the amount of other substances. In our experiment, we used the same ingredients in the same quantity in both groups. Furthermore, CREA showed no differences indicating no kidney lesion occurred.

Better results in terms of reproductive performance in animals supplemented with lipid are probably due to a higher concentration of CHO that should be positively correlated to PG concentration. Accordingly, Ghoreishi et al. (2007) reported higher values of plasma PG and showed a direct correlation between CHO and PG levels (Bianchi et al., 2014). In our trial, no differences in PG levels were found between groups, but a higher correlation between CHO and PG levels was seen in the HPO group compared to the CTR group at d 120. The positive relation between CHO and PG in the HPO group was also detectable after 30 and 60 d and increased up to 120 d, thus suggesting that HPO affects PG synthesis but, at least at the dose of 50 g/(animal·d), was not able to improve reproductive performances in goats.

5. Conclusions

In recent years, the genetic improvement of livestock has brought about high milk production and, therefore, an increase in the energy needed. The addition of lipids in the diet is a tool which provides such nutritive requirements, but possible negative effects on health have been hypothesised. Our results only showed an increase in CHO, and no adverse effects on metabolism were detected. Moreover, despite the relationship between CHO and PG, no effects on reproductive performance were registered.

Finally, biochemical analysis confirmed that the use of 50 g/(animal·d) HPO was tolerated well by lactating dairy goats and that it may be a useful tool to increase energy availability during periods of high demands.

Conflict of interest

We declare that we have no financial and personal relationships with other people or organizations that can inappropriately influence our work, there is no professional or other personal interest of any nature or kind in any product, service and/or company that could be construed as influencing the content of this paper.

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