EFFECTS OF DIFFERENT RUMEN PROTECTED FATS ON SERUM BIOCHEMISTRY AND LIVER ENZYME ACTIVITY IN DORPER SHEEP

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ABSTRACT: *The study was conducted to evaluate the effects of different types of rumen-protected fats (RPF) including prilled fat, prilled fat with lecithin and calcium soap of palm fatty acid on serum biochemistry and serum liver enzyme activities in Dorper sheep. Treatment consisted of basal diet (70:30 concentrate to rice straw) with no added RPF (CON), basal diet plus prilled fat (PF), basal diet plus prilled fat with lecithin (PFL) and basal diet plus calcium soap of palm fatty acids (CaS). Thirty six Dorper sheep were reared at Universiti Putra Malaysia in four treatment groups following completely randomized design. The blood samples were collected in the beginning on day 0 and in the end on day 90 of the experiment to evaluate serum biochemistry parameters and liver enzyme activities. The concentrations of total and HDL, LDL, VLDL cholesterols, glucose, triglycerides and total proteins were neither affected by the diets nor by the sampling days. Moreover, the activity of liver enzymes alkaline phosphatase (ALP), alanine aminotransferase (ALT) and aspartate aminotransferase (AST), was also not influenced. Therefore supplementation of different types of protected fats did not influence serum biochemistry and enzyme activity in Dorper sheep.*

Keywords: Dorper sheep, liver enzyme, rumen-protected fat, serum biochemistry

INTRODUCTION

Rumen-protected fats (RPF) are generally a by-product of palm oil industry, considered as insoluble fats due to their protection from microbial fermentation and biohydrogenation. They remain insoluble at normal rumen pH range of 6 to 7 and escape rumen fermentation. They are then utilized as a source of energy when absorbed through the small intestine [1]. The use of RPF enhance fibre digestibility in high fat supplemented diets by forming insoluble soaps [2]. Hence, RPF can improve the energy density of the diet without affecting rumen fermentation [3].

The calcium soap (calcium salts of palm fatty acids) and the prilled fat are the most extensively used protected fats, both of which are highly digestible. Calcium soap, prilled fat and lecithinized prilled fat are highly concentrated source of energy supplement fats, which are non-hydrogenated and free from trans fatty acids (TFA) specially produced from 100 per cent completely refined palm oil portion. High palmitic acid contents in these fats can bypass rumen and become a direct energy source for the ruminants.

The RPF has been reported to have either significant or non-significant effect on blood serum biochemistry. The blood cholesterol increasing effects of fat supplementation were reported when animals were fed with rumen bypass fat [4, 5]. Another study reported that most saturated fatty acids supplementation increased blood total cholesterol and LDL cholesterol concentrations [6]. The RPF supplementation did not affect serum glucose values in studies conducted by Bhatt et al. [7,8] in lambs, contrary to that an increase was observed in serum glucose with RPF in sheep [9]. However, most of the published studies on sheep have been inconclusive in proving whether the use of protected fats improves serum biochemistry characteristics in sheep could be because of a different age of animals used and method of RPF protection. Therefore, the current experiment was conducted in order to assess the influences of different types of RPF on serum biochemistry including serum cholesterol, glucose, triglycerides, total proteins and liver enzymes activity.

MATERIALS AND METHODS

Location and animals

The experiment was conducted at a ruminant unit of Department of Animal Science, Faculty of Agriculture, Universiti Putra Malaysia (3° 0' 19" North, 101° 42' 12" East) following the guidelines on animal ethics of the Research Policy of Universiti Putra Malaysia. Thirty-six male Dorper sheep about one and a half year of age with an average initial body weight 24.66 ± 0.76 kg (mean ± SE), divided in four groups, kept in individual wooden pens with feeding and drinking facilities were fed with experimental diets for 90 days.

Diets and experimental design

The experiment was carried out using completely randomized design (CRD) and animals were randomly allocated to four treatment groups containing nine animals in each group. The rumen-protected fats (RPF) (Table 1) were obtained from two different companies commercially available in the market. The four isocaloric and isonitrogenous diets, formulated to fulfill the nutritional requirements of sheep as per recommendations of NRC [10], were (1) basal diet (rice straw, corn starch, soybean meal, calcium carbonate, sodium chloride, palm oil, vitamin-mineral mix) with no added RPF (CON); (2) basal diet plus prilled fat (PF); (3) basal diet plus prilled fat with lecithin (PFL); and (4) basal diet plus calcium soap (calcium salt of palm fatty acids) (CaS) on dry matter basis at 5% of the DM (Table 2). The animals were offered calculated feed based on their individual weight at 3% body weight on DM basis.

Table 1 Fatty acid profile of rumen-protected fats

Fatty acids (% of total FA)	PF	PFL	CaS
C15:0	1.39	1.15	1.43
C16:0	72.98	76.72	48.31
C16:1n-9	0.16	0.05	0.81
C18:0	5.16	4.92	4.33
C18:1n-9	16.34	12.85	41.15
C18:2n-6	3.40	3.94	1.64
C18:3n-3	0.57	0.37	2.33
ΣSFA [1]	79.53	82.79	54.07
ΣMUFA	16.5	12.90	41.95
ΣPUFA	3.97	4.31	3.97
n-6:n-3	5.96	10.65	0.70

PF = Prilled fat, PFL = Prilled fat with lecithin, CaS = Calcium soap of palm fatty acids

[1] Calculated ΣSFA = Total saturated fatty acid (C15:0+C16:0+C18:0), ΣMUFA = Total mono-unsaturated fatty acid (C16:1+C18:1), ΣPUFA = Total poly-unsaturated fatty acid (C18:2n-6+C18:3n-3) n-6:n-3 = (C18:2n-6÷C18:3n-3).

Table 2 Ingredients, chemical and fatty acid profile of experimental diets

	Diets					
Ingredient (%)	CON	PF	PFL	CaS	SEM	*P* value
Rice straw (urea treated)	29	33	34	35	--	--
Cornstarch	39	31	30	29	--	--
Soybean meal	26	27	27	27	--	--
Palm oil	4	2	2	2	--	--
Calcium carbonate	1	1	1	1	--	--
Sodium chloride	0.5	0.5	0.5	0.5	--	--
Vitamin-mineral mix [1]	0.5	0.5	0.5	0.5	--	--
PF	--	5	--	--	--	--
PFL	--	--	5	--	--	--
CaS	--	--	--	5	--	--
Total	100	100	100	100	--	--
Chemical composition (% DM)						
Dry matter	91.54	92.08	92.31	91.95	0.176	NS
Organic matter	93.29	91.23	91.31	91.96	0.346	NS
Ether extract	4.98	8.27	8.04	3.38	0.599	*
Crude protein	18.88	18.57	19.92	19.23	0.621	NS
Crude fibre	12.67	28.42	30.09	13.77	2.500	*
Neutral detergent fibre	56.06	58.78	60.22	51.32	1.209	*
Acid detergent fibre	17.89	21.80	24.33	23.94	0.585	NS
Acid detergent lignin	6.65	13.33	10.35	4.92	1.173	*
Metabolizable energy(MJ/kg DM)	11.68	11.65	11.66	11.68	0.274	NS
Fatty acids (% of total FA)						
C15:0	0.78	1.12	0.95	0.60	0.058	*
C16:0	32.93	59.52	61.49	21.21	5.201	NS
C16:1n-9	0.20	0.13	0.11	0.30	0.023	*
C18:0	3.78	4.87	4.58	2.62	0.264	NS
C18:1n-9	42.38	24.20	21.83	52.70	3.867	*
C18:2n-6	18.93	9.69	10.44	21.51	1.560	*
C18:3n-3	1.00	0.47	0.60	1.06	0.077	NS
ΣSFA [2]	37.49	65.51	67.03	24.43	5.508	*
ΣMUFA	42.58	24.33	21.94	53.00	3.890	*
ΣPUFA	19.93	10.16	11.04	22.56	1.636	*
n-6:n-3	18.93	20.62	17.40	20.29	0.445	*

RPF = Rumen protected fat, CON = Basal diet without RPF,

PF = Basal diet + prilled fat, PFL = Basal diet + prilled fat with lecithin,

CaS = Basal diet + calcium soap, '*'= significantly different at (P<0.05), NS = Non-significant,

[1] Contained (g/kg) $ZnSO_4.7H_2O$, 240; CuSO4.5H2O, 70; $MnSO_4$.5H2O, 290; $FeSO_4$.7H2O, 170; (mg/kg) KI, 220; $CoCl2.6H_2O$ 510; NaSeO, 130; pantothenic acid, 750; vitamin B1, 450; vitamin K3, 150; vitamin B12, 0.9; folic acid, 15; vitamin B5, 1,050; vitamin A, 620,000; vitamin D3, 324,000 (IU).

[2] calculated. ΣSFA = Total saturated fatty acid (C15:0+C16:0+C18:0), ΣMUFA = Total mono-unsaturated fatty acid (C16:1+C18:1), ΣPUFA = Total poly-unsaturated fatty acid (C18:2n-6+C18:3n-3) n-6:n-3 = (C18:2n-6÷C18:3n-3)

Sampling of blood

Blood samples were collected on day 0 of the experiment from jugular vein into serum vacutainers and after 90 days of the experiment, during exsanguination when the animals were slaughtered. The blood samples were centrifuged at 4000 g for 15 min [11] and resulting supernatant was collected and stored in centrifuged tubes at -80 ^oC till further analyses.

Serum biochemistry

Blood serum total cholesterol, HDL cholesterol, glucose, triglycerides, total protein, ALT, ALP and AST were analysed using automatic analyzer (Automatic analyzer 902, Hitachi, Germany). The following equation was used to estimate LDL cholesterol [12]:

LDL cholesterol = Total cholesterol - HDL cholesterol – VLDL cholesterol

Where, VLDL cholesterol = Triglycerides / 5

Statistical analysis

Data obtained was analysed using generalized linear model (GLM) of the SAS software (9.4). A factorial design 4 × 2 (diets × days) was employed for serum data. Tukey HSD test was used to separate the means at significance level ($P < 0.05$).

RESULTS

Blood serum biochemistry

There was no significant effect ($P>0.05$) of diet and sampling day found on blood serum biochemistry parameters including concentrations of total cholesterol, LDL cholesterol, HDL cholesterol, VLDL cholesterol, glucose, triglycerides and total proteins with RPF supplementation in Dorper sheep. Inclusion of RPF to the diets (PF, PFL and CaS) numerically increased serum total cholesterol and HDL cholesterol when compared to diet without RPF (CON), as the sampling day progressed from day 0 to day 90, however, RPF supplementation decreased total cholesterol and HDL cholesterol in CON compared to diets with RPF (PF, PFL and CaS) but the difference was not significant ($P>0.05$). The LDL and VLDL cholesterols decreased with RPF supplementation compared to CON on day 90. The VLDL cholesterol increased in CON and PF, remained at the same level in PFL, and decreased in CaS with the inclusion of RPF in the diet as the sampling day progressed from 0 to 90 days. The triglycerides also increased in CON and PF, and decreased in PFL and CaS with supplementation of RPF as the sampling day progressed. No definite trend ($P>0.05$) was observed in serum glucose and serum total proteins in response to the addition of RPF in dietary treatments when compared between sampling days, treatment groups and diet x sampling day interaction .

Table 3 Effects of different types of RPF on serum biochemistry in sheep as influenced by diet and sampling time

Parameter (mmol/L)	Sampling day	Treatments				*P* value	
		CON	PF	PFL	CaS	Diet	Diet x sampling day
Total Cholesterol	0	2.40	1.89	2.23	2.23	0.498	0.518
	90	1.96	2.12	2.46	2.24	0.624	
	P value	0.180	0.455	0.643	0.977		
HDL Cholesterol	0	1.38	1.35	1.47	1.56	0.796	0.754
	90	1.36	1.55	1.79	1.57	0.552	
	P value	0.920	0.388	0.413	0.966		
LDL Cholesterol	0	0.86	0.49	0.61	0.60	0.122	0.266
	90	0.68	0.58	0.62	0.62	0.860	
	P value	0.168	0.386	0.919	0.549		
VLDL Cholesterol	0	0.06	0.04	0.06	0.07	0.346	0.666
	90	0.11	0.05	0.06	0.04	0.580	
	P value	0.563	0.447	0.940	0.128		
Triglycerides	0	0.32	0.19	0.32	0.33	0.3301	0.659
	90	0.53	0.24	0.31	0.21	0.585	
	P value	0.555	0.277	0.942	0.111		
Glucose	0	1.90	1.74	1.62	1.78	0.755	0.976
	90	1.94	1.66	1.52	1.80	0.478	
	P value	0.920	0.774	0.558	0.917		
Total Protein (g/L)	0	52.32	57.56	63.52	64.40	0.140	0.784
	90	47.98	59.00	61.76	58.56	0.076	
	P value	0.561	0.398	0.384	0.459		

CON = Basal diet without RPF, PF = Basal diet + prilled fat, PFL = Basal diet + prilled fat with lecithin, CaS = Basal diet + calcium soap, SEM=standard error of means.

Serum enzyme activity

There was an increase observed in the concentrations of alkaline phosphatase (ALP), alanine aminotransferase (ALT) and aspartate aminotransferase (AST) of sheep fed different rumen-protected fats on day 90 compared to day 0 in all the treatment groups (Figure 1a, 2a and 3a). However, no significant difference ($P>0.05$) was seen when compared to the diets for ALT either on day 0 or on day 90 of the experiment (Figure 1b). The levels of ALP differed significantly ($P<0.05$) between 0 and 90 days (Figure 2a). However, on day 90 there was a significant difference ($P<0.05$) observed in the diet PFL when compared to the diets CON, PF and CaS (Figure 2b). The concentration of AST differed significantly ($P<0.05$) in the diet PFL when compared to the diet CaS on day 90 of the experiment (Figure 3b).

DISCUSSION

Blood serum biochemistry

Diet is one of the important factors which influence blood serum biochemistry profile in ruminants [13]. There was no significant difference seen in blood serum biochemistry parameters including serum cholesterols (total, HDL, LDL and VLDL), triglycerides, glucose and total proteins in sheep fed with rumen-protected fats. There was no sampling day x diet interaction seen in all serum parameters. Total and HDL cholesterol increased in diets containing RPF (PF, PFL and CaS) compared to that in CON diet though the effect is not significant. However, there is a reported evidence of cholesterol increasing effect of fat supplementation in studies reported by Bhatt *et al.* [7]

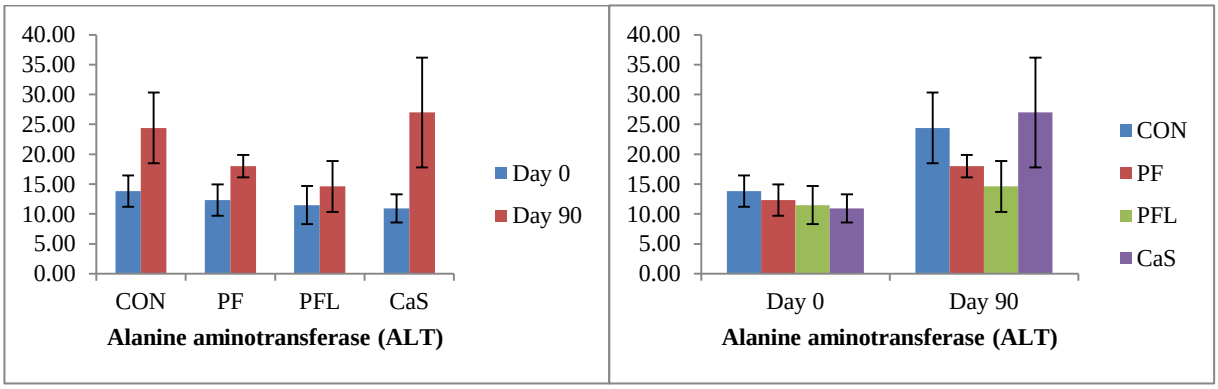

Figure 1 Effect of rumen-protected fats on ALT enzyme activity in Dorper sheep serum (Figure 1a (left)- Comparison between days, Figure 1b (right) – Comparison among treatments) CON = Basal diet without RPF, PF = Basal diet + prilled fat, PFL = Basal diet + prilled fat with lecithin, CaS = Basal diet + calcium soap

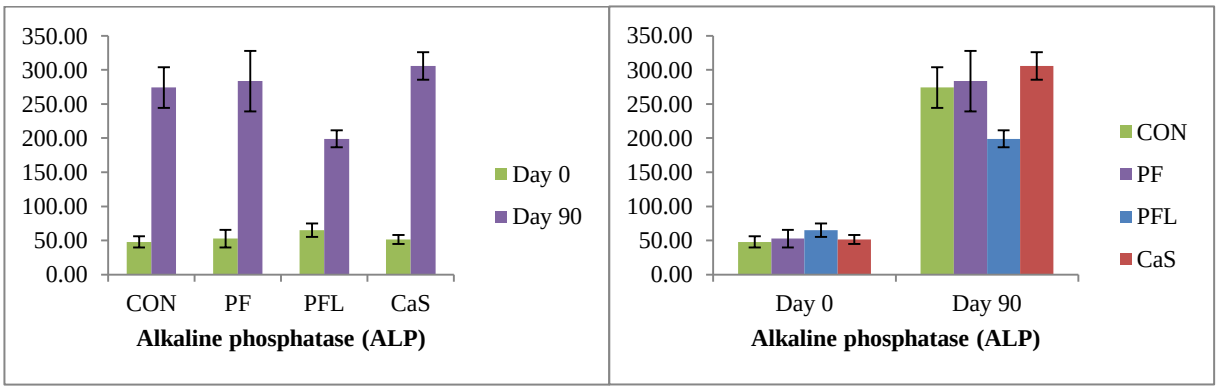

Figure 2 Effect of rumen-protected fats on ALP enzyme activity in Dorper sheep serum (Figure 2a (left)- Comparison between days, Figure 2b (right) – Comparison among treatments) CON = Basal diet without RPF, PF = Basal diet + prilled fat, PFL = Basal diet + prilled fat with lecithin, CaS = Basal diet + calcium soap

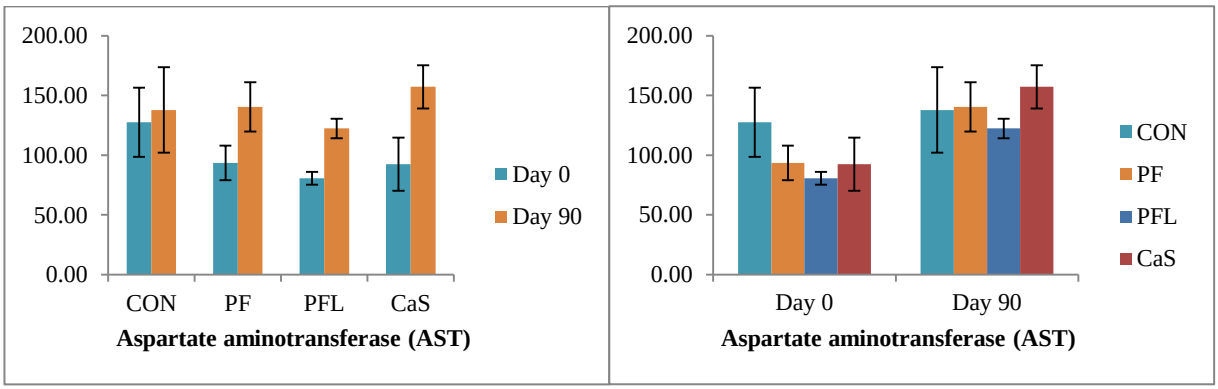

Figure 3 Effect of rumen-protected fats on AST enzyme activity in Dorper sheep serum (Figure 3a (left)- Comparison between days, Figure 3b (right) – Comparison among treatments) CON = Basal diet without RPF, PF = Basal diet + prilled fat, PFL = Basal diet + prilled fat with lecithin, CaS = Basal diet + calcium soap

showed an increase in serum cholesterol concentrations with supplementation of calcium soap of rice bran oil in lambs. A review also suggests that supplementation of larger quantities of RPF increases total fat concentration in plasma and decreases the concentration of LDL [2].

Similarly, either significant or non-significant cholesterol increasing effects of fat supplementation were reported when animals were fed with rumen bypass fat [4;5], dietary tallow and ruminally protected choline [14], whole soybeans and tallow [15], coconut oil [8], palm oil [16], canola oil [17] and blend of palm oil and canola oil [11]. The reason for the increase in serum HDL cholesterol and total cholesterol in the present study could be that the dietary fats stimulated intestinal cholesterol synthesis to fulfil the increased requirements of cholesterol in animals and for digestion, absorption and transport of lipids [18]. As reported by Kott et al. [6] that most saturated fatty acids supplementation increased blood LDL cholesterol and total cholesterol levels. On the contrary, there was a reduction reported in HDL cholesterol and total cholesterol in lambs fed fish meal oil and fish oil mainly because of a marked increase in long-chain n−3 PUFA in the muscle phospholipid fraction [19].

The serum glucose level was not affected significantly (P>0.05) with RPF supplementation but found within normal ranges for healthy sheep [20]. The findings are

supported by Bhatt *et al*. [7;8] who observed similar serum glucose values. In contrast, increased glucose levels were observed with RPF supplementation in ewes [9] because the higher plane of nutrition increased blood glucose at 90 days compared to 0-day values.

There was no significant difference in serum triglycerides and serum total protein levels in the present study. Similar results have been reported by Wadhwa et al. [4] and Tyagi et al.[5], they reported the non-significant influence of RPF supplementation when fed to crossbred cows. Similar results were reported by Adeyemi et al. [11] and Li *et al*. [21], with supplementation of dietary oils. In contrast to that, dietary supplementation of RPF improved triglycerides level ($P<0.01$) in the diets with RPF supplemented group compared with control group [4]. Another study also found increased triglyceride levels by feeding sunflower or soybean oil compared to control group [13].

Blood serum enzyme activity

Aspartate aminotransferase (AST) is an enzyme having high activity in the liver [22]. Increased activity of AST in the serum is a sensitive indicator of liver damage. Alkaline phosphatase (ALP) enzyme activity in ruminants increases in case of hepatitis and biliary disorders [23]. It is also influenced by nutrition [24]. Alanine aminotransferase (ALT) is an enzyme of liver, and its upsurge in the blood is specific for modifications in the liver [25]. Determination of enzyme activities of AST, ALT and ALP in blood serum would be useful in identifying liver functions as these enzymes are sensitive indicators of hepatocellular injury, thus their elevations can indicate the presence of liver dysfunction [26].

The activities of ALT, ALP and AST were not influenced by the diet. However were significantly ($P<0.05$) influenced by sampling day in sheep fed with rumen-protected fats. Statistically, the concentrations of liver enzymes were similar throughout the treatment groups, thus were not modified by RPF supplementation. The diet PFL had numerically the lowest concentration of ALT, ALP and AST compared to other treatment groups for day 90. Similar findings were observed by Rahmani et al. [27] who reported that feeding choline did not significantly ($P>0.05$) affect activities of ALT, AST and GGT in dairy cows but there was a significant difference between the groups for ALP levels.

In contrast to the present study, Parvar *et al*. [28] reported that liver enzymes ALP, AST and ALT were modified by dietary treatments in lambs supplemented with different concentrations and combinations of canola oil, fish oil and soybean oil. Similar findings were observed by Bianchi et al. [29] stating that activity of AST and GGT increased significantly with supplementation of soybean oil fed at 6% of dry matter in sheep. The chief functions of the liver are metabolism of fat, carbohydrate and protein. Scarpino et al. [30] reported that increased AST levels in animals supplemented with oil may indicate liver damage which is ascribed to liver weight loss. In the present study, values for ALT, ALP and AST were 22-38, 70-390 and 60-280 units/L respectively, which are within the normal ranges for sheep [20]. The results suggest that the activities of ALT, ALP and AST were not significantly influenced by RPF supplementation. Therefore, supplementation of rumen-protected fats at present concentration 5% did not cause any untoward effect on the liver. However, RPF supplementation has decreased the activity of ALT, ALP and AST insignificantly in animals fed with the diet containing prilled fat with lecithin (T3) compared to other diets.

CONCLUSION

The supplementation of rumen-protected fats in the form of prilled fat, prilled fat with lecithin and calcium soap did not influence serum cholesterols (total, HDL, LDL and VLDL), triglycerides, glucose and total proteins significantly in comparison to the diet without RPF (CON). Moreover, the activity of liver enzymes ALT, ALP and AST were also not affected by RPF supplementation. The values of these enzymes in the current experiment were within the normal range for sheep. Therefore supplementation of different types of protected fats did not influence serum biochemistry and enzyme activity in Dorper sheep.

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