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The Effects of Diets Containing Tallow and Cotton Seed Oil on Liver and Serum Parameters in Fattening Bulls

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Abstract: The effects of addition of fats to the diet on some liver and serum parameters of fattening bulls were examined in breeding conditions. Twenty-four Brown Swiss calves, average weight of 275 kg, were assigned to three groups and fed the following mixed feeds for 183 days: I, control without added fat (CON); II, 2.5% vegetable oil contained (CSO); III, 2.5% animal fat contained (TAL). In the study, the activities of aspartate aminotransferase (AST), alanine aminotransferase (ALT), gamma-glutamyltransferase (GGT) and lactate dehydrogenase (LDH) were measured. The serum concentrations of total protein (TP), albumin, glucose, cholesterol, triglyceride, urea, creatinine, calcium, phosphorus, magnesium, sodium and potassium and the concentration of liver glycogen and protein were determined. This study showed that both at the end of the backgrounding and the finishing period, differences for enzyme activities of AST, ALT, GGT and LDH, concentrations of TP, albumin, creatinine, Ca, P, Mg, Na and K among the groups were not statistically significant. However, glucose values were significantly higher ($P<0.05$) in TAL in both periods. Urea and P were significantly lower in vegetable oil in the backgrounding period while cholesterol and tryglyceride concentrations were significantly lower with vegetable oil in the finishing period. On the other hand, feeding bulls on the diet including animal fat caused a significant increase of the liver glycogen level ($P<0.01$). Liver protein was not influenced by fat addition.

Key words: Animal fat, vegetable oil, liver, blood, bull

Introduction

Added fats have been used successfully for in poultry, swine rations and cattle rations. Added fat can help to cover the energy demands of cattle. Common fat sources include oilseeds and animal fat, and various ruminally inert fat products. Cottonseed oil and animal fat are often the cheapest sources of fat; therefore there is great interest in maximizing their utilization in diets for cattle (Schauff *et al.,* 1992). Whole oilseeds are less likely to interfere with rumen fermentation than fats because they are slowly digested in the rumen and therefore slowly release the oil (Palmquist and Jenkins, 1980). Tallow is often called saturated fat, but about 50% of its fatty acids are unsaturated (Chalupa *et al.,* 1986). Several studies have reported that depression of cellulose and protein digestibility is most severe for fat sources high in unsaturated fatty acids, which inhibit growth and function of ruminal microbes more than saturated fatty acids (Palmquist and Jenkins, 1980; Whitney *et al.,* 2000). Feed fats are hydrolyzed to free fatty acids and glycerol and the unsaturated fatty acids are hydrogenated rapidly (Olubobokun *et al.,* 1985; Chalupa *et al.,* 1986; Jenkins and Fotouhi, 1990; Kucuk *et al.,* 2001). The majority of the studies on fat addition to cattle diets have been carried out mainly with concentrate-forage based diets fed to dairy cattle. However, several studies have investigated the effects of feeding either vegetable oil or tallow to fattening cattle. Furthermore, there are a few reports that evaluated the effects of different fat additions on blood metabolites (Lammoglia *et al.,* 1999). As cottonseed oil and animal fats often are considered the cheapest sources of fat compared to the other energy sources, nutritionists could suggest to breeders using them on cattle diets how the effects of diets containing fats were not known clearly on blood metabolites and liver. Research is needed to determine the effect of adding fat to beef rations on liver and serum parameters. Therefore, the aim of this study was to investigate the probable effects of feeding diets containing vegetable oil and animal fat on liver and serum parameters in fattening bulls reared under intensive fattening conditions.

Materials and Methods

Experimental design: Twenty-four "Brown Swiss" male cattle with an average live weight of 275 kg, selected from a commercial fattening farm, were used in this study. Animals were randomly allotted by group of eight to one of three dietary treatments: 1) control without contained fat (CON), 2) 2.5% cottonseed oil contained (CSO), 3) 2.5% tallow contained (TAL). Animals were adapted to the treatment diets within 2 weeks.

Animal maintenance and nutrition: The bulls were fed in open lot pens. All breeding conditions were the same for all groups of animals during the experimental period in commercial fattening farm. The intensive fattening period was 183 days (backgrounding period=day 0-56, finishing period=day 57-183). All animals were fed with a fixed amount of wheat straw and concentrate diets and water ad libitum. Each group was consumed 3.760 kg DM/day in backgrounding period, 1.250 kg DM/day in finishing period. The composition of the concentrate diets was determined by the methods of Weende analyses (AOAC, 1990). Samples of concentrate diets were analyzed individually and the average chemical composition is given in Table 1. The temperature and relative humidity in total fattening period were between 5.6 and 24.9°C, between 39% and 96% respectively.

Table 1: Ingredient and chemical composition of experimental diets

Ingredient, % (air-dry basis)	CON	CSO	TAL
Maize	15.45	5.1	5.1
Barley	42.0	44.88	44.88
Wheat bran	24.75	25.72	25.72
Sunflower meal	12.92	14.66	14.66
Molasses	1.0	2.0	2.0
Limestone	2.38	3.64	3.64
Vitamine - Mineral premix[1]	0.1	0.1	0.1
Sodium bicarbonate	1.0	1.0	1.0
Salt	0.4	0.4	0.4
Fat	-	2.5	2.5
Analysed nutrient contents, % air-dry basis			
Crude protein	13.02	13.49	13.49
Crude fiber	10.67	10.62	10.64
Ether extracts	2.41	3.92	3.91
Metabolizable energy, kcal/kg	2752	2790	2788

[1]Provided per kg diet: Vit. A 15000 IU, Vit D_3 3000 IU, Vit E 30mg/kg, Mn 50mg/kg, Zn 50mg/kg, Fe 50mg/kg, Cu 10mg/kg, I 0.8mg/kg, Co 0.15mg/kg, Se 0.15mg/kg

Measurements and analyses

Analysis of blood samples: Approximately 5 ml of blood was collected into sterile glass tubes via jugular vein puncture of each animal at initial and end of the back grounding and finishing period. The blood was allowed to clot for 24 h at 4°C. Serum was obtained by centrifugation (1700 x g for 10 minutes) and frozen at 20°C until it was analyzed. Serum enzyme activities of aspartate aminotransferase (AST), alanine aminotransferase (ALT), gamma-glutamyltransferase (GGT), lactate dehydrogenase (LDH); serum concentrations of total proteins (TP), albumin, glucose, cholesterol, tryglyceride, urea, creatinine, calcium (Ca), phosphorus (P) and magnesium (Mg) were analyzed spectrophotometrically (Microlab 200, Merck, Deutschland) using a commercial reagent kits (Biomedical Systems, Barcelona, Spain). The concentrations of sodium (Na) and potassium (K) were measured using ion selective electrode (Ion selective, Easy lite, England).

Analysis of liver samples: At the end of the experiment, all animals were slaughtered at a commercial abattoir after 8 h fasting. After slaughter, liver sample from slaughtered each animal was collected at the abattoirs. Stainless surgical blades were used to cut off the liver samples. The samples (4-5 g) were transferred into glass tubes with plastic cover that contains ice-cold trichloroacetic acid (%10 TCA) and immediately frozen at -20 C until analysed. The quantity of glycogen in the liver was measured enzymatically (Nicholas *et al.*, 1956; Joseph *et al.*, 1961). Protein concentrations were measured by the method of Lowry *et al.* (1951) with cattle serum albumin (BSA) as standard. All analyses were run in triplicate and the mean values are reported.

Statistical analysis: The data were analyzed according to the analysis of variance procedure using the general linear model in SPSS (1997). The model was designed to determine the effect of feed treatment on liver and serum parameters. The statistical differences between means were examined by Duncan's multiple range tests.

Results

Measured values at the end of backgrounding and finishing period for serum enzyme activities, protein, metabolite, mineral and electrolyte values are given in Table 2. In the backgrounding period; differences for enzyme activities of AST, ALT, GGT and LDH, serum concentrations of TP, albumin, cholesterol, tryglyceride, creatinine, Ca, Mg, Na and K among the treatment groups were not statistically significant. Glucose values were significantly higher ($P<0.05$) in TAL as compared with CON and CSO, but differences between CON and CSO were not statistically significant. The values of Urea and P were significantly lower in CSO when compared with CON and TAL. However, no statistical significant differences in the values urea and P were found between CON and CSO.

In the finishing period; differences for enzyme activities of AST, ALT, GGT and LDH, serum concentrations of TP, albumin, creatinine, Ca, P, Mg, Na and K among the treatment groups were also not statistically significant. The values of glucose were significantly higher in TAL than the other groups ($P<0.05$), but the difference between CON and CSO was not statistically significant. The values of cholesterol and tryglyceride concentrations were significantly higher in CSO when compared with their respective mean concentrations in CON and TAL. Urea value was significantly lower in CSO, but the difference between CON and TAL was not statistically significant.

Liver glycogen and protein values are given in Table 3.

Table 2: Serum parameters in treatment groups

Parameters	Period	CON Mean±SEM	CSO Mean±SEM	TAL Mean±SEM	P	Referens values[a,b]
Enzymes						
AST, U/l	BP	101.3±5.4	107.9±5.6	112.4±7.4	0.45	78-132
	FP	102.8±6.2	110.6±4.5	104.5±6.7	0.62	
ALT, U/l	BP	24.5±2.6	23.3±1.9	23.6±2.2	0.92	90-170
	FP	20.6±1.6	23.6±2.2	20.3±1.9	0.42	
GGT, U/l	BP	14.0±1.1	12.8±.1.1	14.9±1.0	0.38	11-24
	FP	14.0±1.0	13.8±.1.0	12.9±0.9	0.70	
LDH, U/l	BP	147.5±5.2	131.4±18.9	141.9±10.1	0.67	8-302
	FP	154.5±8.5	158.4±5.9	161.9±6.3	0.76	
Proteins						
Total proteins, g/dl	BP	5.6±0.2	5.5±0.2	5.9±0.2	0.40	6.7-7.5
	FP	5.8±0.2	5.7±0.2	5.7±0.2	0.97	
Albumin, g/dl	BP	3.4±0.1	3.2±0.2	3.2±0.2	0.53	3.0-3.6
	FP	3.3±0.2	3.4±0.1	3.6±0.1	0.19	
Metabolites						
Glucose, mg/dl	BP	63.3^{b}±6.7	69.6^{b}±6.9	93.8^{a}±3.6	0.004	45-75
	FP	77.6^{b}±5.4	76.4^{b} ±3.8	94.9^{a}±3.6	0.01	
Cholesterol, mg/dl	BP	111.5±6.2	130.1±9.5	138.9±8.4	0.08	80-180
	FP	112.9^{b}±5.0	129.3^{b}±8.8	156.6^{a}±10.2	0.004	
Tryglyceride, mg/dl	BP	12.75±0.8	12.1±0.8	14.5±1.1	0.17	0-14
	FP	13.8^{b}±0.8	11.0^{b}±0.6	19.3^{a} ±1.4	0.001	
Urea, mg/dl	BP	32.3^{a}±2.4	24.6^{b}±1.6	33.6^{a}±1.6	0.01	25-35
	FP	31.9^{a}±2.2	23.4^{b}±2.2	28.2^{a}±2.3	0.04	
Creatinine, mg/dl	BP	1.2±0.1	1.5±0.2	1.1±0.1	0.21	1-2
	FP	1.2±0.1	1.3±0.1	1.5±0.1	0.15	
Minerals and electrolytes						
Calcium, mg/dl	BP	9.5 ±0.23	9.4±0.3	9.7±0.3	0.60	9.7-12.4
	FP	9.5 ±0.31	9.5±0.4	8.9±0.2	0.38	
Phosphorus, mg/dl	BP	4.9^{a} ±0.2	4.8^{b} ±0.2	5.5^{a}±0.1	0.03	4.6-6.5
	FP	5.1 ±0.2	5.3 ±0.2	5.1 ±0.2	0.68	
Magnesium, mg/dl	BP	1.8±0.1	1.8±0.1	1.8±0.1	0.93	1.4-2.3
	FP	1.8±0.1	1.7±0.1	1.7±0.1	0.93	
Sodium, meq/l	BP	137.3±0.9	136.6±1.3	138.8±1.0	0.39	132-152
	FP	140.6±1.5	139.1±0.6	138.1±0.5	0.21	
Potassium, meq/l	BP	4.5±0.1	4.4.±0.1	4.6±0.1	0.59	3.9-5.8
	FP	4.5.±0.1	4.3±0.1	4.5±0.1	0.30	

[a,b]:Means within rows for each characteristics with no common superscript differ (P<0.01). SEM: Standard error of mean. BP: backgrounding period FP: finishing period, AST: aspartate aminotransferase, ALT: alanine aminotransferase, GGT: gamma-glutamyltransferase LDH: actate dehydrogenase. [a]referens values were taken from Altintas and Fidanci (1993), [b]Turgut, (2000)

Although significant difference was found for liver glycogen between treatments (P<0.01), there was no significant difference for liver protein. Liver glycogen was significantly lower in CON while it was higher in TAL compared to CSO.

Discussion

When serum parameters were examined in Table 2, significant differences were not found among the diet groups for enzyme activities of AST, ALT, GGT and LDH, proteins of TP and albumin, metabolites of cholesterol, tryglyceride and creatinine, minerals and electrolytes of Ca, Mg, Na, K. There were not significant differences between CON and CSO for glucose, but TAL had a higher glucose concentration compared to the other treatment groups. The probable explanation for the higher glucose value in TAL is the long transit time of animal fat in the rumen as related to digestibility time in rumen, rumination time, chewing count, ruminal pH, dry matter, free fatty acids and ADF due to physical and chemical characteristics of animal fat. This interpretation is supported by the findings of several researchers (Jenkins and Fotouhi, 1990; Lafond *et al.*, 2001; Nelson *et al.*, 2001). Besides, with increasing transit time in rumen, it could be thought that blood values reach their peak later. There were no significant differences between CON and TAL for urea and P values. Although, the values of urea and P were found lower in CSO, it was considered that there was no obvious effect resulting from feeding because these values were within the normal range (Altintas and Fidanci, 1993). At the end of the finishing period, significant differences were not found among the treatment groups for enzyme activities of AST, ALT, GGT and LDH, proteins of TP and albumin,

Table 3: Liver glycogen and protein in treatment groups

Parameters	n	CON	CSO	TAL	P
		Mean±SEM	Mean±SEM	Mean±SEM	
Glycogen, mg/g tissue	24	4.3^{c}±0.6	6.1^{b}±0.3	11.6^{a}±0.7	0.001
Protein, mg/g tissue	24	155.7±14.0	145.2±6.8	130.7±5.7	0.20

^{a,b,c}: Means within rows for each characteristics with no common superscript differ significantly (P<0.01).

metabolites of creatinine, minerals and electrolytes of Ca, P, Mg, Na, K. There was no significant difference in glucose between CON and CSO while TAL had higher glucose values compared to the other treatments. This may be explained by the idea that blood values reach the peak later because of a longer transit time of animal fat in the rumen. Especially, this effect may be explained by the obvious increase of glucose in TAL compared to the other treatments due to higher concentrate consumption in the finishing period and cooler climate and hard digestibility of animal fat (Jenkins and Fotouhi, 1990; Lafond *et al.*, 2001; Nelson *et al.*, 2001). Significant differences were not determined for urea between CON and TAL, but CSO had lower urea values than the other groups. However all values were within the normal range (Altintas and Fidanci, 1993).

Tryglyceride and cholesterol values were higher in TAL compared to CON and CSO. Higher tryglyceride in TAL and cholesterol values depend on the fat source consumed. Although, it is considered that animal fats have higher proportion of saturated fats and these fats lead to an increase in cholesterol in the organism (Wrenn *et al.*, 1979; Talavera *et al.*, 1991), cholesterol values were within the normal range (Altintas and Fidanci, 1993; Turgut, 2000). In this study it is also observed that fat added diets had no detrimental effects on liver because enzyme values (indicate liver function) and mineral, urea and creatinine values (indicate kidney functions) were within the normal range at the both feeding period.

Liver glycogen was higher in TAL and CSO than in CON. This finding is consistent with the other reports (Fluharty and Loerch, 1997; Lammoglia *et al.*, 1999; Schoonmaker *et al.*, 2003). These researchers reported that feeding supplemental fat to animals can results in the increase of glycogen because greater concentrations of carbohydrates are stored as glycogen in all vertebrates. Liver protein values were non-significantly different between the diets but numerically higher in CON as compared to those in TAL and CSO. Liver protein was lower in TAL compared to CSO. In the case of feed deficiency and other stress factors, it was reported that synthesis of liver plasma protein might increase (Powanda *et al.*, 1980; Dibner and Ivey, 1990). Fatty acids inhibit microorganism in the rumen, even inhibitory effect of on unsaturated fatty acids lead to the formation of stress factors and all of them cause an increase in liver protein value as has been reported by Palmquist and Jenkins, (1980). Higher liver protein in CON may have been caused by accumulation of protein in liver. However, further research is needed to determine the relationship between ruminants liver parameters and feeding.

Conclusion: The results of this study show that the effects of the addition of cottonseed oil and tallow in fattening farm conditions were different on blood parameters and liver. The temperature and relative humidity in total fattening period were between 5.6 and 24.9°C, between 39% and 96% respectively. Differences were observed for glucose, cholesterol and tryglyceride values in blood parameters caused by fat source treatment. Furthermore, the tallow addition to diets of fattening bulls in an intensive fattenning system increased the liver glycogen at the end of the backgrounding and finishing period. It could be said that 2.5% cottonseed oil and tallow supplementation to diets of fattening bulls has no detrimental effects on liver functions.

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