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Acyl Interchange with Enzymes Promises a New Route to Tailored Properties in Fats

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High premium fats and oils often exhibit a specific arrangement of saturated and unsaturated acids on glycerol. Their unique properties are due in large part to whether a specific type of fatty acid (saturated or unsaturated) is attached to a primary (terminal) or secondary (middle) position of the glycerol. Melting points of fats and oils can be increased by hydrogenation but hydrogenation gives no control as to the position of attachment and, therefore, does not provide a route to those unique thermophysical properties due to the fatty acid position. An objective of this project was to explore the selective interchange of these fatty acids as a means of upgrading tallow for special applications.

Synthetic methods for producing specific types of triglycerides are indeed known. These generally involve the preparation of an equilibrated 1,2- and 1,3-diglyceride mixture from available triglyceride and separation of the isomeric diglycerides, followed by acylation with fatty acid anhydride or chloride. In addition to requiring the conversion of fatty acid to anhydride or chloride, relatively high temperatures are required. Another approach to tailored properties is the fractionation of beef tallow into a hard fat, a cocoa butter-like semisolid and an oil. This approach is limited only by the types and amounts of triglycerides available in the fat chosen for fractionation.

The scope of availability of premium property fats and diglycerides could be further expanded in view of more recent research at the Eastern Regional Research Center of the U.S.D.A.

While the projected equilibrium yields in SUS are encouraging, further yield of SUS could be expected in any process in which SUS is removed to shift the equilibrium to the right. Also, while the mono- and diglycerides produced might themselves show properties commanding a premium, there appear to be possibilities for producing more premium triglycerides from them. One of these would be anhydride esterification, which has the disadvantage of preparing and using fatty acid anhydride. However, since 1,2-diglyceride is produced by this lipase, it does not require a separation of 1,2- and 1,3-diglycerides.

Another reaction of potential use in converting a 1,2-diglyceride, SU-OH, to SUS is based on a direct acyl interchange reaction in a diester system, i.e., $2SU\text{-}OH \rightleftarrows SUS + HO\text{-}U\text{-}OH$, with lipase at 8.0 pH. Recently the preparation and use of immobilized lipase for the hydrolysis of triglycerides was reported. These immobilized enzymes are less subject to inactivation than dissolved enzymes and thus can provide long-term catalysis at low cost. Their use for reesterification would further expand the scope of the glyceride reactions discussed. For $2SU\text{-}OH \rightarrow SUS + HO\text{-}U\text{-}OH$, the use of immobilized lipase could possible be the basis of an anhydrous reaction. Here the enzyme support itself would form with the oil phase (diglyceride or diglyceride/solvent) the interface required for lipase action. An immobilized lipase could also be useful in the hydrous system for $SU\text{-}OH + S \rightarrow SUS + H_2O$ and $HO\text{-}U\text{-}OH + 2S \rightarrow SUS + 2H_2O$ and in fact for the primary S/SUU equilibration process itself.

Recent research has shown that syntheses of SUS from SUU and S is feasible in a low temperature reaction catalyzed by lipase. By-product mono- and di-glycerides which by themselves may be useful as digestive adjuvants are potentially convertible to additional SUS by anhydride esterification or more usefully by lipase catalysis. Developments in immobilized lipase are promising and suggest the availability of a long-lived catalyst for these reactions. The potential of a low temperature, low cost process for premium property triglycerides could provide a route to these materials from readily available depressed market fats and oils. While the reaction $SUU + S \rightarrow SUS + U$ has been used as an example here, analogous reasoning should apply to the replacement of any primary acyl group in any triglyceride.

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THE EFFECT OF FATS AND UREA ON IN VITRO CELLULOSE DIGESTION[1,2]

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Summary

A closed in vitro system (24 hr digestion period) was used with a two factorial randomized block design to study the effect of added corn oil (5 and 7%) and tallow (5 and 7%) to wood cellulose (Solka-Floc) with three levels of urea (.234, 2.34 and 4.68%) expressed as percent of cellulose. All fat levels resulted in lower dry matter disappearance (DMD) when no urea was added compared to negative controls with neither fat nor urea. Adding urea at 2.34 and 4.68% significantly ($P<.01$) increased DMD with all fat levels but to a lesser extent with 7% tallow. Ammonia N values were higher ($P<.01$) when 7% tallow and 4.68% urea were added together. Total volatile fatty acids (TVFA) were depressed ($P<.01$) for 7% corn oil and 5 and 7% tallow. Reduction of TVFA was primarily due to the reduction of acetic acid. Gross energy from TVFA was lowest for 7% tallow for every level of urea.

Introduction

Nonprotein nitrogen (NPN), primarily in the form of urea, is widely used in ruminant feedlot rations because urea generally can supply nitrogen at a lower cost than from plant proteins. The ability of microflora in the rumen to convert NPN to amino acids and protein may receive more attention as the worlds population continues to grow and competition for protein increases. When economics permit, tallow may also be used in feedlot rations to increase caloric density and aid in controlling dust.

Several studies have been done using fat and urea in combination in feedlot rations. Although results are somewhat variable, several researchers have reported depressed gains and feed efficiency (Jones et al., 1961; Bradley et al., 1966; Thompson et al., 1967; Church et al., 1971; Hatch et al., 1972). Crude fiber has been shown to be one of the fractions with reduced digestibility when fat and urea are fed together (Embry et al., 1957).

This study was conducted to study the effects of adding corn oil and tallow with urea on cellulose digestiln in vitro and the production of ammonia, volatile fatty acids (VFA) and gross energy from VFA's.

Materials and Methods

A closed in vitro system was used with a randomized block design and a 5 x 4 factorial arrangement of treatment was used in the experiments. Wood cellulose (Solka-Floc) was used as the substrate and the variable treatments were 0 fat, 5 and 7% corn oil or 5 and 7% tallow and urea levels of 0, .234, 2.34 or 4.68% urea expressed as percent of substrate. Rumen fluid was collected from a fistulated steer maintained on a 50% grain supplement and 50% alfalfa ration. Twenty ml of a 1:1 mixture of rumen fluid to McDougall buffer were added to .5 g of substrate. Urea was dissolved in water and mixed so that each flask received 10 ml. Incubation was at 39 C for 24 hours. After incubation, each flask was filtered through a Gooch crucible and the filtered material was dried at 100 C for 24 hrs and used in dry matter disappearance (DMD) determination. A zero time control was used to correct for dry matter in the rumen fluid. Liquid samples obtained at filtering were used for ammonia and VFA procedures. VFA's were determined using aqueous solutions in a gas liquid chromatograph fitted with a flame ionization detector. Ammonia-nitrogen (NH_3-N) was determined using an aeration method described by Hawk et al. (1954). Gross energies produced from VFA's were calculated using values reported by Blaxter (1962).

Analysis of variance using least squares analysis (Steel and Torrie, 1960) was used to determine differences in dry matter disappearance, ammonia nitrogen, volatile fatty acids and gross energy from volatile fatty acids. Means were tested for significance at the ($P<.05$) and ($P<.01$) levels.

Results and Discussion

The effects of fat and urea on dry matter disappearance (DMD) are shown in Table 1. Addition of urea at the 2.34 or 4.68% level resulted in significant ($P<.01$) increases in DMD and the addition of either corn oil, 5 or 7%, or tallow, 5 or 7%, resulted in a ($P<.01$) depression in DMD. Although DMD was less for 7% corn oil than for 5%, the differences were not significant. Differences between respective levels of corn oil and tallow showed no significant differences in DMD. Greatest depression within the fat-urea treatments was observed with 4.68% urea and 7% tallow. This reduction in DMD was significant ($P<.01$) when compared to 4.68% urea and 5% tallow.

Ammonia nitrogen (NH_3-N) values are shown in Table 2. There were no significant differences in NH_3-N between zero and .234% urea in any fat treatment but the addition of 2.34 or 4.68% urea resulted in increases in NH_3-N ($P<.01$). Tallow added at 5 or 7% resulted in significant increases ($P<.05$) in NH_3-N. The greatest values for NH_3-N were found in the 7% tallow treatment. With the 7% tallow treatment and 2.34 and 4.68% urea NH_3-N values were increased ($P<.01$) when compared to no fat controls. None of the other fat treatments differed significantly.

Other researchers (Robertson and Hawke, 1964, and Thompson et al., 1967) have reported increased ruminal ammonia due to feeding oils or fat and urea in combination. Increased NH_3-N values with in-

[1]Oregon Agricultural Experiment Station Technical Paper No. 4231.

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TABLE 3. VOLATILE FATTY ACIDS (VFA) CONCENTRATIONS AFTER 24 HOUR DIGESTION.

	Cellulose + No fat - no urea	Cellulose + .23% urea	Cellulose + 2.3% urea	Cellulose + 4.7% urea
Total VFA, μm/ml	63.96[a]	64.71[a]	71.11[b]	76.43[c]
C_2, %	62.52	62.57	60.54	58.94
C_3, %	25.02[a]	24.78[a]	27.48	29.22[b]
C_4, %	12.46	12.70	11.98	11.84
	Cellulose + 5% C.O - no urea	Cellulose + 5% C.O + .23% urea	Cellulose + 5% C.O + 2.3% urea	Cellulose + 5% C.O + 4.7% urea
Total VFA, μm/ml	67.17[a]	64.91[a]	74.21[b]	75.08[b]
C_2, %	61.99	61.53	61.43	61.12
C_3, %	25.22	25.51	27.34	28.26
C_4, %	12.79	12.96	11.23	10.62
	Cellulose + 7% C.O - no urea	Cellulose + 7% C.O + .23% urea	Cellulose + 7% C.O + 2.3% urea	Cellulose + 7% C.O + 4.7% urea
Total VFA, μm/ml	59.60[a]	64.62[b]	69.05[c]	66.28[bc]
C_2, %	62.27	61.47	59.07	58.31
C_3, %	24.66[a]	25.92[a]	29.65[b]	30.07[b]
C_4, %	13.07	12.61	11.28	11.62
	Cellulose + 5% tallow - no urea	Cellulose + 5% tallow + .23% urea	Cellulose + 5% tallow + 2.3% urea	Cellulose + 5% tallow + 4.7% urea
Total VFA, μm/ml	52.75[a]	64.47[b]	71.63[c]	61.38[b]
C_2, %	61.21	63.27	60.30	59.08
C_3, %	25.04[a]	24.76[a]	27.71	29.11[b]
C_4, %	13.75	11.97	11.99	11.81
	Cellulose + 7% tallow - no urea	Cellulose + 7% tallow + .23% urea	Cellulose + 7% tallow + 2.3% urea	Cellulose + 7% tallow + 4.7% urea
Total VFA, μm/ml	49.87[a]	61.46[b]	62.11[b]	58.98[b]
C_2, %	63.04	62.22	60.20[a]	64.36[b]
C_3, %	23.88	24.23	26.97[a]	23.04
C_4, %	13.08	13.55	12.83	12.60

[a,b,c]Means on the same line with different superscripts differ significantly (P<.05).

TABLE 4. GROSS ENERGY FROM VOLATILE FATTY ACIDS (cal/ml).

Fat level	Urea level, % None	.234	2.34	4.68
No fat	18.43[ae]	18.66	20.66	22.38[be]
5% corn oil	19.44[ae]	18.85	21.37	21.58[e]
7% corn oil	17.25	18.74	20.14	19.45
5% tallow	15.41[ad]	18.45	20.84[b]	17.96[d]
7% tallow	14.38[ad]	17.84	18.16[b]	16.84[d]

[a,b] Means on the same line with different superscripts differ significantly (P<.05).
[d,e] Means in the same column with different superscripts differ significantly (P<.05).