

AN ABSTRACT OF THE THESIS OF

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Title: EFFECT OF DIFFERENT NITROGEN SOURCES ON MOLASSES-BASED LIQUID SUPPLEMENTS FOR CATTLE. *Redacted for Privacy*

Abstract approved: _____

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The potential use of hair meal, feather meal, single cell protein from pulp mill secondary clarifiers and liquefied fish as supplementary sources of nitrogen in molasses based liquid supplements, was studied in palatability as well as in vitro and in vivo digestion trials.

For the palatability and in vitro digestion trials, 5, 10, 15 and 20% dry matter from the supplemental nitrogen sources were suspended in molasses and urea was added to make all the liquid supplements iso-nitrogenous with 30% crude protein. Three supplements were made with 5% of either single cell protein, hair or feather meal, plus 5% of liquefied fish, while two supplements had urea as the supplemental nitrogen source with molasses or liquefied grain starch as the principal energy sources.

Ten yearling heifers were used for the palatability trial. Rye-grass straw and liquid supplement were provided ad libitum and intake was monitored daily on individual animals. The diet had higher analytical crude protein with the feather meal than single cell protein

supplements ($P < .01$), while the gross energy content was higher with liquefied fish and single cell protein than hair meal and feather meal supplements ($P < .01$). Dry matter and energy consumption per kg body weight (BW) were higher for liquefied fish and hair meal ($P < .01$), but energy consumption per kg $BW^{.75}$ was higher for the hair meal supplements ($P < .01$).

The in vitro trials were conducted using ryegrass straw as substrate and liquid supplements were added to increase the crude protein up to 19.54%. In vitro dry matter disappearance (IVDMD) increased linearly while ammonia production showed a hyperbolic response as liquid supplements were added to the substrate ($P < .05$). Acetic to propionic acid ratio decreased as liquid supplements were added to the media ($P < .05$). Regression equations are reported to predict IVDMD, ryegrass straw, liquid supplement and total dry matter intake from the parameters measured.

Fifteen crossbred Hereford heifers were used in the in vivo digestion trial to quantify the effect of additional crude protein (0, 1, 2, 3 and 4 g/kg $BW^{.75}$) on voluntary intake and apparent digestibility of the nutritional components of the diet. The basal ration used was ryegrass straw. In the liquid supplements the crude protein sources supplied 10% of the supplemental nitrogen as feather meal or liquified fish (trial 1) or hair meal (trial 2). The feather meal liquid supplement promoted higher consumption of straw, dry matter, crude protein and digestible energy (kg or g/kg $BW^{.75}$, and Mcal or

or Kcal/kg BW^{.75}, respectively; P<.05). Straw consumption was increased by the addition of 2 g of crude protein/kg BW^{.75} in trial 1 (P<.01), and total dry matter and crude protein consumptions were improved by the addition of 1 g of crude protein/kg BW^{.75} from the three liquid supplements under study (P<.01). Digestible energy consumption was improved at the third and first level of crude protein supplementation (trial 1 and 2, P<.01 and P<.05; respectively). Nevertheless, the experimental animals never achieved a positive energy balance. Concentration of urea in blood plasma increased at the highest level of protein supplementation (P<.01 and P<.05; trials 1 and 2, respectively). Total ruminal fatty acid production increased at the second and first level of supplemental crude protein for trial 1 and 2, respectively (P<.05 and P<.01) and changed to a propionate pattern of fermentation as the diet protein was increased. This change decreased the acetate:propionate ratio (P<.01 and P<.05; trial 1 and 2, respectively). The liquefied fish liquid supplements had a detrimental effect on dry matter and gross energy digestibilities (P<.05), while the feather and hair meal supplements improved them (P<.05 and N. S., respectively). Crude protein digestibility was highly improved at the first level of nitrogen supplementation with the three liquid supplements under study (P<.01). Crude protein supplementation was the parameter that showed the highest correlation coefficients with consumption and digestibility, and was also the most important factor to predict consumption under the experimental conditions of this work.

Effect of Different Nitrogen Sources on Molasses-based Liquid
Supplements for Cattle

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CHAPTER I

Effect of different nitrogen sources on molasses-based liquid supplements for cattle. 1. Palatability and in vitro digestion trials¹.

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SUMMARY

Palatability and in vitro digestion trials were conducted in order to evaluate the effectiveness of single cell protein from pulp mill secondary clarifiers , hair meal, feather meal, liquefied fish and urea to optimize dry matter intake and in vitro digestion on molasses-based liquid supplements. Five, 10, 15 and 20% of dry matter from the supplemental nitrogen sources were suspended in molasses and urea was added to make all the mixtures isonitrogenous with 30% crude protein. Three supplements were made with 5% of either single cell protein, hair or feather meal plus 5% of liquefied fish , and two supplements had only urea as the supplemental nitrogen source and molasses or liquefied grain starch as the principal energy sources. Ten yearling heifers were used for the palatability trial. Ryegrass straw and liquid supplements were provided ad libitum and intake was monitored daily on individual animals. The diet had higher analytical crude protein with feather meal than the single cell protein supplements ($P < .01$), while the gross energy content was higher with the liquefied fish and single cell protein than hair and feather meal supplements ($P < .01$). Dry matter and energy consumption per kg of body weight (BW) were higher for liquefied fish and hair meal ($P < .01$), but energy consumption per kg BW^{.75} was higher for the hair meal supplements ($P < .01$).

The in vitro trials were conducted using ryegrass straw as substrate and liquid supplements were added to increase the crude protein up to 19.54%. In vitro dry matter disappearance (IVDMD) increased linearly while ammonia production showed a hyperbolic response as liquid supplements were added to the substrate ($P < .05$). Acetic to propionic

acid ratio decreased as liquid supplements were added to the media ($P < .05$). Regression equations are reported to predict IVDMD, ryegrass straw, liquid supplement and total dry matter intake from the parameters measured.

INTRODUCTION

The liquid industry has grown considerably during the past few years. Some advantages as well as disadvantages of liquid over dry feeds were published previously (Anonymous, 1969).

Total dry matter intake of poor quality roughages is enhanced by the addition of protein (Elliot, 1967; Church, 1979; Santos, 1979) but this addition can have a detrimental effect on intake if highly digestible roughages are offered (Blaxter et al., 1961; Campling and Murdoch, 1966). The control of supplement intake has been a problem. Incorporation of salt in dry supplements is by far the most common method used to restrict intake without detrimental effect on performance, provided that abundant water is available (Cardon et al., 1951; Nelson et al., 1955). Liquid supplement intake has been controlled with the use of calcium chloride (Rapp and Anderson, 1956), salt (Templeton et al., 1970), phosphoric acid, and restricted feeding (Anonymous, 1969). No improvement on dry matter intake was found with liquid supplements when urea was the sole source of nitrogen (Phillips and Vavra, 1979), but Hennesy et al. (1978) reported an increase in digestible organic matter intake with the same kind of supplements. Incorporation of true protein into the supplements has improved performance in some instances (Velloso et al., 1971).

It is commonly accepted that in vitro digestion techniques are an important tool to evaluate animal feeds. In a recent publication (Menke et al., 1979), in vitro techniques were used to predict digestibility and metabolizable energy content of feeds with a coefficient of determination (r^2) of .95. In vitro dry matter digestibility accounted for 67% of the variation in dry matter intake by sheep (Walters, 1971). In vitro dry matter disappearance was also enhanced with isocaloric and isonitrogenous liquid supplements (Ortega et al., 1980). The sole source of nitrogen in these trials was urea, while the crude protein content of the supplements ranged from 20 to 70%. The substrates utilized were cellulose or ryegrass straw.

The present series of trials were planned to investigate the optimum level of different non-protein nitrogen (besides urea) and true protein sources in molasses-based liquid supplements. Supplement palatability, straw and total dry matter intake by cattle and in vitro dry matter disappearance of ryegrass straw were considered in evaluating supplements.

MATERIALS AND METHODS

Molasses-based isonitrogenous (30% CP) liquid supplements were mixed where urea furnished more than 75% of the nitrogen needed. The remainder of the nitrogen was supplied by either feather meal (FM; 5-03-795), hair meal (HM), single cell protein from pulp mill secondary clarifiers (SCP) and liquified fish prepared from whole hake (LF; Law, 1973).

Palatability trial.

This trial was designed to determine the optimum amount of supplemental source of nitrogen (mentioned above) needed to control acceptability and maximize ryegrass straw (1-04-059) consumption by yearling heifers.

Twenty one liquid supplements were tested in this trial. Sixteen of these supplements were prepared by suspending 5, 10, 15 or 20% dry matter (DM) from HM, FM, SCP or LF in molasses. Another three LS were prepared with 5% LF plus either 5% HM, FM or SCP, respectively. Urea was added to each combination to bring the mixtures to 30% CP. For the 20th supplement, all the supplemental nitrogen was from urea and ammonium polyphosphate (table 1). Another supplement was included where the principal carbohydrate source was liquefied grain starch and the nitrogen source was urea (LGS)¹.

Five Hereford and five Holstein yearling heifers were used to evaluate the acceptability of these liquid supplements by cattle. Three, 5 x 5 latin square designs were used for these purposes, and the SCP, HM or FM liquid supplements were tested in each of the squares. Within each square all the animals were allowed to consume all the liquid supplements. A 5 x 6 x 5 (heifer x supplement x day) factorial arrangement was used to test the supplements with LF, LGS and (or) urea. Ryegrass straw was supplied ad libitum and consumption was monitored for each animal daily. Each heifer had access to only one of the supplements for four days and daily consumption was also recorded. All the animals had water and trace mineralized salt free choice. Feed samples were taken to analyze for

¹Kemin Industries, Inc., Des Moines, IA 50301.

TABLE 1. COMPOSITION OF LIQUID SUPPLEMENTS

Supplement ^a	Composition, %						Analytical Data			
	SCP	HM	FM	LF	Urea	Molasses	Water	DM, %	CP, % ^b	Energy, Mcal/kg ^b
SCP5	3.64				9.05	69.19	18.11	57.61	27.06	2.26
SCP10	7.42				8.60	66.78	17.20	59.74	28.73	2.38
SCP15	11.34				8.13	64.27	16.26	64.16	29.08	2.40
SCP20	15.42				7.64	61.66	15.28	66.54	27.06	2.50
SCP5-LF5	3.95			18.23	6.76	71.06		60.08	25.13	2.00
HM5		3.75			8.33	71.25	16.66	60.60	30.31	2.02
HM10		7.88			7.05	70.96	14.10	63.55	28.91	2.19
HM15		12.47			5.63	70.64	11.27	66.61	30.62	2.38
HM20		17.57			4.05	70.28	8.11	69.72	31.57	2.39
HM5-LF5		3.98		18.38	6.01	71.63		63.85	26.30	2.16
FM5			3.75		8.36	71.19	16.72	62.41	25.18	2.31
FM10			7.87		7.12	70.79	14.23	69.13	34.03	2.60
FM15			12.42		5.74	70.36	11.48	67.55	26.03	2.62
FM20			17.47		4.21	69.89	8.43	71.37	28.88	2.70
FM5-LF5			3.98	18.38	6.04	71.61		64.34	25.52	2.13
LF5				18.14	7.23	74.62		61.96	25.64	2.36
LF10				31.98	5.70	62.32		56.09	22.81	2.22
LF15				42.89	4.50	52.61		49.68	20.42	1.96
LF20				44.77	3.52	51.70		48.68	21.16	2.23
UREA ^c					8.25	76.60	11.60	58.26	26.85	1.75
LGS ^d								42.96	44.77	1.88
Ryegrass straw								92.43	5.91	4.00

^aAll supplements had .01% KELFLO (suspending agent). Kelco, Division of Merck & Co., Inc.

^bAs fed basis

^cPlus 3.25% ammonium polyphosphate

^dLiquefied grain starch. Kemin Industries, Inc.

DM, CP and gross energy (GE) (AOAC, 1975). With these data, total DM, CP and GE consumptions were calculated. Least square analysis of variance was performed for 5 x 5 latin square and factorial designs to quantify the variance of total consumption of CP and GE as well as consumption per unit of weight (BW) or metabolic body weight ($BW^{.75}$) (Gill, 1978a, 1978b). Stepwise regression analyses were also conducted to see which nitrogen source and proximate analysis component had the greatest effect on DM intake (Neter and Wasserman, 1974).

In vitro trials

All the supplements used for the palatability trial were tested in in vitro digestion trials. Ryegrass straw was the substrate used as well as the negative control. Different amounts of the different supplements were added in order to cover all the nutritional regimens achieved by the experimental animals in the palatability trial. The treatments tested ranged from 6.03 (CP content of the ryegrass straw, DM basis) to 19.54% CP for each of the supplements (tables 5 to 8). All treatments combinations were conducted in triplicate in seven runs. Rumen liquor was collected from a yearling rumen-fistulated steer that had received 1 kg/d of a mixture of the 21 supplements mentioned above. Ryegrass straw was supplied ad libitum along with water and trace mineralized salt. Twenty ml of a 1:1 mixture of rumen fluid to McDougall's buffer (McDougall, 1948) were added to .5 g treatment preparations, flushed with CO₂ and incubated at 39 C for 24 hours. After incubation, each sample was filtered through a preweighed Gooch crucible and dried at 100 C overnight to determine in vitro dry matter disappearance (IVDMD). Liquid samples obtained from filtering were used for ammonia-N

(IVNH3-N) and volatile fatty acid (VFA) determinations. Ammonia-N was determined using an aeration method described by Hawk et al. (1954). Samples were prepared for VFA determination by a method similar to that described by Baumgardt (1964) and the analysis conducted on a gas liquid chromatograph². Least square analysis of variance was conducted to detect differences due to supplements and levels (21 x 5 x 3 factorial arrangement) (Gill, 1978a, 1978b). Regression analysis was done to find the optimum level of supplemental nitrogen source in the substrate to achieve the highest IVDMD (Neter and Wasserman, 1974).

RESULTS

The experimental animals used in the palatability trial chose different nutritional regimens (table 2); the diet had higher CP content when FM was the supplemental source of nitrogen than when the SCP was included ($P < .01$). The energy content of LF and SCP diets were higher than for the HM and FM treatments ($P < .01$). There was no treatment effect on DM consumption (kg), CP consumption/kg BW^{.75} or total GE consumption (Mcal). Animals consumed more of the FM supplements, resulting in more total CP consumption ($P < .01$). The CP consumption with the FM supplements was not different ($P > .01$) than the CP intake when HM was the supplemental source of nitrogen, while the supplemental energy consumption was the highest for the former treatment ($P > .01$).

The different supplemental nitrogen sources had an effect on straw

²Varian-Aerograph (model 1200). Varian-Aerograph Co., Walnut Creek, CA.

TABLE 2. SUMMARY OF NUTRITIONAL REGIME ACHIEVED BY EXPERIMENTAL ANIMALS IN THE PALATABILITY TRIAL

<u>Item</u>	<u>SCP</u>	<u>HM</u>	<u>FM</u>	<u>LF</u>
<u>CP in diet, %</u>	16.22 ^b	17.96 ^{bc}	20.10 ^c	18.17 ^{bc}
<u>GE in diet, Mcal/kg</u>	4.18 ^c	4.06 ^b	4.08 ^b	4.18 ^c
<u>Dry Matter</u>				
Total cons., kg/day	8.19	7.87	7.90	7.12
Supp. cons., kg/day	2.15 ^b	2.35 ^b	3.09	2.15 ^b
Straw cons., kg/day	6.05 ^c	5.52 ^{bc}	4.81 ^b	4.97 ^b
Total cons., % BW/day	1.93 ^b	2.60 ^c	1.82 ^b	2.37 ^c
Total cons., % BW ^{.75} /day	8.77 ^b	10.84 ^c	8.30 ^b	9.86 ^{bc}
<u>Crude Protein</u>				
Total cons., kg/day	1.337 ^a	1.416 ^a	1.599	1.275 ^a
From supp., kg/day	.951 ^b	1.063 ^{bc}	1.292 ^c	.957 ^b
From straw, kg/day	.387 ^c	.353 ^{bc}	.307 ^b	.318 ^b
Total cons., g/kg BW/day	3.16 ^b	4.67 ^d	3.69 ^{bc}	4.22 ^{cd}
Total cons., g/kg BW ^{.75} /day	14.34	19.47	16.82	17.58
<u>Gross Energy</u>				
Total cons., Mcal/day	34.15	31.97	32.22	29.87
From supp., Mcal/day	7.97 ^b	8.07 ^b	11.41	8.34 ^b
From straw, Mcal/day	26.18 ^c	23.90 ^{bc}	20.82 ^b	21.53 ^b
Total cons., Kcal/kg BW/day	80.60 ^b	105.68 ^c	74.22 ^b	99.44 ^c
Total cons., Kcal/kg BW ^{.75} /day	365.53 ^{bc}	440.46	338.68 ^b	413.78 ^c

^aMeans in the same row with the same superscript are not different (P<.05)

^{bcd}Means in the same row with the same superscript are not different (P<.01)

consumption and, consequently, on CP and GE consumption from straw ($P < .01$). Total DM and GE consumption, expressed as a percentage and Kcal/kg of BW, respectively, were higher for LF and HM ($P < .01$). The CP consumption/kg BW followed the same tendency, but the consumption of cattle on the LF treatments was not different than those on the FM treatments ($P > .01$). When consumption was expressed as a percentage of $BW^{.75}$, the differences in CP consumption disappeared ($P > .01$) while GE consumption was greater for HM than SCP or FM ($P < .01$).

The comparisons of the average nutrient consumption among and within the SCP, HM and FM treatments are shown in table 3. In this case the only differences found were in regards to supplement consumption (kg/d, as fed) ($P < .05$), supplemental CP and GE consumptions, total CP, CP/kg BW and $CP/kg BW^{.75}$ consumptions and CP and GE content of the diet chosen ($F < .01$). Supplement consumption was greater when 20% FM was suspended in molasses than for supplements SCP10, SCP15, SCP 20, HM5 and HM10 ($P < .05$). Supplemental CP consumption was higher for FM10 than all the SCP levels, HM5, HM10 and FM5 ($P < .01$). The later tendency was also followed for total CP consumption, except that SCP5 and SCP20 were not different than FM10 ($F > .01$). CP intakes per kg BW and per $kg BW^{.75}$ were higher for HM20 than SCP5, SCP10, SCP15, SCP20, FM5 and FM5LF5, and all of the above but SCP5 and FM5LF5, respectively ($P < .01$). The highest intake of CP was achieved with FM10 and the lowest GE intake with supplement FM5LF5 ($P < .01$). The addition of 5% LF to 5% SCP or HM consistently improved consumption. However, when FM was the main supplemental nitrogen source, this improvement was not observed. The LF treatments were

TABLE 3. NUTRITIONAL REGIMENS ACHIEVED BY EXPERIMENTAL ANIMALS IN THE LIQUID SUPPLEMENT PALATABILITY TRIAL WHEN SCP, HM, AND FM WERE USED AS SUPPLEMENTAL N-SOURCES

Supplement	Supplement Consumption			Total CPCons, kg	CP Consumption		CP, %	G.E., Mcal/kg
	As fed, kg	C.P., kg	G.E., Mcal		g/kg BW	g/kg BW.75		
SCP5	3.87 ^{abcd}	1.048 ^{ef}	8.74 ^{efg}	1.47 ^{ef}	3.44 ^{efg}	15.62 ^{efg}	16.70 ^{efg}	4.225 ^{1J}
SCP10	2.74 ^a	.788 ^e	6.54 ^e	1.12 ^e	2.64 ^e	11.96 ^e	16.29 ^{ef}	4.249 ^J
SCP15	3.17 ^{abc}	.923 ^{ef}	7.61 ^{efg}	1.29 ^e	3.08 ^{ef}	13.91 ^{ef}	16.56 ^{efg}	4.175 ^{hi}
SCP20	3.25 ^{abc}	.879 ^e	8.14 ^{efg}	1.29 ^{ef}	3.05 ^{ef}	13.83 ^{ef}	14.79 ^e	4.192 ^{hi}
SCP5LF5	4.43 ^{abcd}	1.114 ^{efg}	8.85 ^{efg}	1.52 ^{ef}	3.62 ^{efgh}	16.38 ^{efg}	16.73 ^{efg}	4.036 ^{efg}
HM5	3.02 ^{ab}	.915 ^{ef}	6.09 ^e	1.27 ^e	4.18 ^{efgh}	17.43 ^{efg}	17.28 ^{efg}	4.081 ^{fg}
HM10	3.15 ^{abc}	.910 ^{ef}	6.90 ^{ef}	1.26 ^e	4.18 ^{efgh}	17.41 ^{efg}	16.84 ^{efg}	4.095 ^B
HM15	3.73 ^{abcd}	1.142 ^{efg}	8.86 ^{efg}	1.50 ^{ef}	4.96 ^{gh}	20.66 ^{fg}	18.53 ^{efg}	4.097 ^B
HM20	3.95 ^{abcd}	1.248 ^{efg}	9.43 ^{efgh}	1.58 ^{ef}	5.19 ^h	21.66 ^B	19.81 ^{fg}	4.016 ^e
HM5LF5	4.19 ^{abcd}	1.103 ^{efg}	9.05 ^{efg}	1.47 ^{ef}	4.83 ^{gh}	20.17 ^{fg}	17.34 ^{efg}	4.031 ^{ef}
FM5	3.67 ^{abcd}	.924 ^{ef}	8.48 ^{efg}	1.18 ^e	2.73 ^e	12.46 ^e	18.61 ^{efg}	4.104 ^B
FM10	4.90 ^{cd}	1.666 ^B	12.72 ^{gh}	1.97 ^f	4.53 ^{fgh}	20.69 ^{fg}	24.34	4.091 ^{fg}
FM15	4.63 ^{bcd}	1.206 ^{efg}	12.14 ^{fgh}	1.51 ^{ef}	3.47 ^{efg}	15.85 ^{efg}	18.69 ^{efg}	4.158 ^h
FM20	5.17 ^d	1.493 ^{fg}	13.94 ^h	1.83 ^{ef}	4.22 ^{efgh}	19.24 ^{efg}	20.39 ^B	4.104 ¹
FM5LF5	4.58 ^{abcd}	1.169 ^{efg}	9.74 ^{efgh}	1.50 ^{ef}	3.48 ^{efg}	15.85 ^{efg}	18.46 ^{efg}	3.958

^{abcd} Means in the same column with the same superscript are not different (P < .05)

^{efghIJ} Means in the same column with the same superscript are not different (P < .01)

compared between them and against the UREA and LGS supplements. No difference was detected within the levels of LF in the supplements for the parameters reported except in the case of GE consumption, where LF20 ranked the highest. UREA and LGS treatments consistently ranked low when compared to the supplements that had LF as the supplemental nitrogen source ($P < .05$ and $P < .01$, table 4).

All the liquid supplements were incubated with rumen liquor using ryegrass straw as a substrate; results have been summarized in tables 5 to 8. Differences due to supplements and level of supplement were detected for all the parameters measured ($P < .05$) except for production of isobutyric acid. The lowest IVDMD were for the negative controls in runs 1 and 6, 6.97 and 7.04 CP levels for LF10 and UREA with mean values of 19.34, 20.21, 18.90 and 21.36% IVDMD, respectively. Higher IVDMD was attained with 17.0 and 17.59% CP for SCP5 and HM20 with values of 42.46 and 41.75%, respectively ($P < .05$).

Ammonia production showed a hyperbolic response. The negative controls had the lowest IVNH₃-N, while the higher CP levels had higher values with a range of .022 to .501 mg/ml for the negative control (run 2) and with FM5 (17.1% CP), respectively ($P < .05$). The range of total VFA was from 139.72 to 1,024.82 $\mu\text{mol/ml}$ for negative control in run 1 and 16.44% CP level with FM10, respectively ($P < .05$). These VFA production values were lower and higher, respectively, than the remainder of the treatments tested ($P < .05$). Acetic (C2) and butyric (C4) acid production tended to decrease while propionic (C3) increased with increases of liquid supplement level in the substrate. This was reflected in the C2:C3

TABLE 4. NUTRITIONAL REGIMENS ACHIEVED BY EXPERIMENTAL ANIMALS IN THE PALATABILITY TRIAL WHEN LF, UREA AND LGS WERE THE SUPPLEMENTAL N-SOURCES UNDER STUDY

Supplement	Supplement consumption			Total CP Cons, kg	CP consumption, g		GE, Mcal/kg
	As fed, kg	CP, kg	GE, Mcal		/kg BW	/kg BW ^{.75}	
LF5	3.93 ^{cd}	1.01 ^{cd}	9.26 ^d	1.31 ^{ab}	4.37 ^{ab}	18.18 ^{ab}	4.13 ^d
LF10	5.61 ^d	1.28 ^d	12.45 ^d	1.62 ^b	5.18 ^b	21.77 ^b	4.19 ^d
LF15	4.76 ^d	.97 ^{cd}	9.31 ^d	1.28 ^{ab}	4.27 ^{ab}	17.77 ^{ab}	4.20 ^d
LF20	5.37 ^d	1.14 ^{cd}	11.97 ^d	1.49 ^{ab}	4.81 ^{ab}	20.14 ^{ab}	4.41
UREA	2.23 ^c	.60 ^c	3.91 ^c	.87 ^a	2.93 ^a	12.14 ^a	4.00 ^c
LGS	1.86 ^c	.83 ^{cd}	3.50 ^c	1.22 ^{ab}	4.15 ^{ab}	17.17 ^{ab}	4.26 ^d

^{ab}Means in the same column with the same superscript are not different (P<.05)

^{cd}Means in the same column with the same superscript are not different (P<.01)

TABLE 5. EFFECT OF ADDED CP FROM SINGLE CELL PROTEIN TO RYEGRASS STRAW ON RUMEN FERMENTATION *IN VITRO*

Treatment	CP ¹	GE ²	SUPPEN ³	IVDMD ⁴	IVNH3 ⁵	TOTVFA ⁶	C2 ⁷	C3 ⁷	C4 ⁷	C5 ⁷	I ⁷	A/P ⁸
Neg Control Run 1	6.03	4.07		19.3 ^a	.023 ^a	139 ^{a+c}	64 ^c	19 ^a	12 ^{a+c}	1.1	2.2	3.33 ^{j,k}
SCP5 1	7.56	4.06	3.5	31.9 ^{g+t}	.032 ^a	254 ^{a+c}	61 ^{b,c}	23 ^{b,c}	10 ^{a,b}	1.8	1.9	2.60 ^{b+j}
2	9.15	4.06	7.2	32.0 ^{h+u}	.066 ^{a+d}	242 ^{a+c}	63 ^{b,c}	23 ^{a+k}	10 ^{a,b}	1.4	1.5	2.68 ^{b+j}
3	12.39	4.05	14.8	39.1 ^{x+z}	.177 ^{a+l}	481 ^{a+d}	60 ^{a+c}	26 ^{d+q}	9 ^{a,b}	1.6	1.2	2.24 ^{a+h}
4	17.0	4.03	25.7	42.4 ^z	.320 ^{m+t}	317 ^{a+d}	60 ^{a+c}	29 ^{l+r}	8 ^a	1.2	.6	2.08 ^{a+g}
Neg Control Run 2	6.03	4.07		25.9 ^{b+l}	.022 ^a	295 ^{a+c}	57 ^{a,b}	26 ^{c+q}	12 ^{a+c}	1.7	1.6	2.21 ^{a+h}
SCP10 1	7.66	4.07	3.9	28.3 ^{c+m}	.042 ^{a,b}	226 ^{a+c}	59 ^{a+c}	26 ^{c+q}	11 ^{a,b}	1.5	1.2	2.28 ^{a+h}
2	9.32	4.06	8.0	30.4 ^{f+o}	.058 ^{a+d}	281 ^{a+c}	58 ^{a+c}	27 ^{i+q}	11 ^{a,b}	1.4	1.1	2.11 ^{a+g}
3	12.25	4.06	15.1	34.6 ^{m+x}	.205 ^{a+m}	860 ^{a+c}	60 ^{b,c}	27 ^{f+g}	10 ^{a,b}	.8	.4	2.26 ^{a+h}
4	16.66	4.05	25.9	38.3 ^{v+z}	.365 ^{n+t}	447 ^{a+d}	56 ^{a,b}	29 ^{n+r}	11 ^{a,b}	1.6	1.0	1.93 ^{a+d}
SCP15 1	7.34	4.06	3.0	22.4 ^{a+c}	.045 ^{a,b}	371 ^{a+d}	58 ^{a+c}	25 ^{b+o}	12 ^{a,b}	2.1	1.8	2.33 ^{a+l}
2	8.97	4.04	6.8	31.6 ^{h+s}	.073 ^{a+e}	261 ^{a+c}	61 ^{b,c}	25 ^{b+q}	10 ^{a,b}	1.7	1.1	2.38 ^{a+l}
3	11.76	4.02	13.4	33.3 ^{j+w}	.189 ^{a+m}	224 ^{a+c}	61 ^{b,c}	26 ^{c+q}	10 ^{a,b}	1.4	.8	2.36 ^{a+l}
4	16.36	3.98	24.4	39.4 ^{y+z}	.365 ^{o+t}	285 ^{a+c}	58 ^{a+c}	29 ^{m+r}	9 ^{a,b}	1.4	.8	2.01 ^{a+f}
SCP20 1	7.73	4.06	4.1	28.8 ^{c+m}	.040 ^a	329 ^{a+d}	57 ^{a,b}	27 ^{h+q}	11 ^{a,b}	1.8	1.9	2.09 ^{a+g}
2	9.31	4.04	7.9	31.0 ^{f+q}	.068 ^{a+e}	230 ^{a+c}	60 ^{a+c}	26 ^{e+q}	10 ^{a,b}	1.3	1.2	2.26 ^{a+h}
3	12.22	4.02	15.0	33.8 ^{i+x}	.220 ^{d+o}	338 ^{a+d}	59 ^{a+c}	26 ^{d+q}	12 ^{a,b}	1.0	.5	2.22 ^{a+h}
4	16.47	3.99	25.6	38.5 ^{w+z}	.318 ^{d+s}	289 ^{a+c}	59 ^{a+c}	30 ^{q,r}	8 ^{a,b}	1.1	.5	1.94 ^{a+d}
Neg Control Run 3	6.03	4.07		24.2 ^{a+e}	.054 ^{a+c}	328 ^{a+d}	62 ^{b,c}	23 ^{a+k}	10 ^{a,b}	2.0	1.2	2.62 ^{b+j}
SCP5LF5 1	6.82	4.05	1.7	24.4 ^{a+f}	.096 ^{a+g}	306 ^{a+c}	58 ^{a+c}	25 ^{b+q}	11 ^{a,b}	2.0	1.5	2.27 ^{a+h}
2	8.70	4.02	5.8	26.4 ^{b+l}	.133 ^{a+j}	410 ^{a+d}	58 ^{a+c}	24 ^{a+n}	11 ^{a,b}	2.4	2.2	2.38 ^{a+l}
3	11.61	3.96	12.3	30.9 ^{f+g}	.270 ^{j+q}	255 ^{a+c}	60 ^{a+c}	25 ^{b+p}	11 ^{a,b}	1.7	1.1	2.41 ^{a+l}
4	16.07	3.87	22.6	38.9 ^{x+z}	.469 ^u	333 ^{a+d}	58 ^{a+c}	30 ^{p+r}	8 ^{a,b}	1.8	.5	1.96 ^{a+e}

abcdefghijklmnopqrstuvwxy. Means in the same column with the same superscript across tables 5 to 8 are not different (P<.05).

¹CP = Crude protein, %

²GE = Gross energy, Mcal/kg

³SUPPEN = GE from supplement, %

⁴IVDMD = *In vitro* dry matter disappearance, %

⁵IVNH3 = *In vitro* ammonia production, mg/ml

⁶TOTVFA = Total volatile fatty acid production, μ mol/ml

⁷C2,C3,C4,C5,I = Acetate, propionate, butyrate, valerate and isomers of butyrate and valerate, respectively, % moles

⁸A/P = C2:C3 ratio

TABLE 6. EFFECT OF ADDED CP FROM HAIR MEAL TO RYEGRASS STRAW ON RUMEN FERMENTATION *IN VITRO*

Treatment	CP ¹	GE ²	SUPPEN ³	IVDMD ⁴	IVNH3 ⁵	TOTVFA ⁶	C2 ⁷	C3 ⁷	C4 ⁷	C5 ⁷	I ⁷	A/P ⁸
Neg Control Run 3	6.03	4.07		24.2 ^{a,e}	.054 ^{a,c}	328 ^{a,d}	62 ^{b,c}	23 ^{a+k}	10 ^{a,b}	2.0	1.2	2.62 ^{h+j}
HM5 1	7.20	4.05	2.3	24.2 ^{a,e}	.089 ^{a,g}	257 ^{a,c}	62 ^{b,c}	23 ^{a+l}	10 ^{a,b}	1.4	1.9	2.59 ^{a+f}
2	9.01	4.02	5.9	28.2 ^{c,m}	.140 ^{a,k}	260 ^{a,c}	62 ^{b,c}	23 ^{a+l}	11 ^{a,b}	1.8	1.1	2.72 ^{h+k}
3	11.85	3.96	11.8	29.6 ^{f,o}	.264 ^{i,p}	309 ^{a,c}	59 ^{a,c}	25 ^{b+p}	11 ^{a,b}	2.1	1.6	2.34 ^{a+l}
4	16.62	3.88	22.0	37.9 ^{s,z}	.375 ^{n,u}	455 ^{a,d}	55 ^{a,b}	31 ^r	10 ^{a,b}	1.8	.8	1.73 ^a
HM10 1	7.14	4.05	2.4	25.5 ^{b,h}	.075 ^{a,f}	372 ^{a,d}	58 ^{a,c}	26 ^{c,q}	11 ^{a+c}	1.9	1.7	2.22 ^{a+h}
2	8.88	4.02	6.3	27.7 ^{b+l}	.117 ^{a+j}	318 ^{a,d}	61 ^{b,c}	24 ^{a+l}	10 ^{a,b}	1.7	1.6	2.58 ^{a+f}
3	11.62	3.98	12.5	32.2 ^{i,v}	.203 ^{a,m}	410 ^{a,d}	59 ^{a,c}	26 ^{c,q}	10 ^{a,b}	1.6	1.3	2.27 ^{a+h}
4	15.98	3.91	22.6	38.0 ^{s,z}	.377 ^{p,u}	560 ^{a,d}	56 ^{a,b}	29 ^{n+r}	11 ^{a,b}	1.8	1.0	1.89 ^{a+c}
Neg Control Run 4	6.03	4.07		24.5 ^{a,f}	.073 ^{a,f}	344 ^{a,d}	61 ^{a,c}	21 ^{a+c}	12 ^{a+c}	2.4	2.5	2.87 ^{f+k}
HM15 1	7.02	4.06	2.3	26.0 ^{b+i}	.077 ^{a,f}	253 ^{a,c}	57 ^{a,b}	26 ^{d,q}	11 ^{a,b}	2.2	2.5	2.21 ^{a+h}
2	8.65	4.03	6.1	29.5 ^{d+n}	.092 ^{a,g}	262 ^{a,c}	58 ^{a,c}	23 ^{a+l}	13 ^{a+c}	2.2	1.8	2.46 ^{a+l}
3	10.07	4.02	9.4	29.5 ^{e+o}	.183 ^{a,m}	287 ^{a,c}	59 ^{a,c}	24 ^{a+n}	13 ^{a,c}	1.5	1.0	2.46 ^{a+l}
4	15.00	3.95	21.4	37.1 ^{r+z}	.336 ^{n+t}	414 ^{a,d}	60 ^{a,c}	27 ^{i,q}	9 ^{a,b}	1.3	1.4	2.24 ^{a+h}
HM20 1	7.72	4.04	3.9	27.6 ^{b+k}	.055 ^{a,c}	257 ^{a,c}	62 ^{b,c}	23 ^{a+k}	11 ^{a,b}	1.4	1.4	2.65 ^{b+j}
2	11.06	3.97	13.0	32.4 ^{j+w}	.118 ^{a+j}	153 ^a	64 ^{b,c}	22 ^{a+f}	10 ^{a,b}	1.6	1.6	2.95 ^{g+k}
3	13.93	3.93	19.1	35.6 ^{n+x}	.182 ^{a,m}	347 ^{a,d}	57 ^{a,b}	25 ^{b+q}	13 ^{a,b}	1.7	1.5	2.24 ^{a+h}
4	17.59	3.86	28.4	41.7 ^z	.281 ^{k+q}	268 ^{a,c}	56 ^{a,b}	27 ^{f+q}	12 ^{a,b}	1.8	2.3	2.07 ^{a+f}
HMSLF5 1	7.34	4.05	2.8	25.2 ^{b,h}	.048 ^{a,c}	187 ^{a,b}	63 ^{b,c}	21 ^{a+c}	11 ^{a,b}	1.4	2.1	3.02 ^{h+k}
2	9.03	4.01	6.6	29.1 ^{d+n}	.108 ^{a+f}	245 ^{a,c}	57 ^{a,b}	25 ^{b+q}	13 ^{a+c}	2.0	1.6	2.28 ^{a+h}
3	11.70	3.97	12.7	31.6 ^{g+r}	.188 ^{a,m}	389 ^{a,d}	56 ^{a,b}	25 ^{b+q}	13 ^{b,c}	2.0	2.1	2.18 ^{a+h}
4	16.07	3.88	23.0	38.1 ^{t+z}	.397 ^{p,u}	371 ^{a,d}	55 ^{a,b}	29 ^{o+r}	11 ^{a,b}	1.2	.6	1.87 ^{a,b}

abcdefghijklmnopqrstuvwxyz Means in the same column with the same superscript across tables 5 to 8 are not different (P<.05).

¹CP = Crude protein, %

²GE = Gross energy, Mcal/kg

³SUPPEN = GE from supplement, %

⁴IVDMD = *In vitro* dry matter disappearance, %

⁵IVNH3 = *In vitro* ammonia production, mg/ml

⁶TOTVFA = total volatile fatty acid production, μ mol/ml

⁷C2,C3,C4,C5,I = Acetate, propionate, butyrate, valerate and isomers at butyrate and valerate, respectively, % moles

⁸A/P = C2:C3 ratio

TABLE 7. EFFECT OF ADDED CP FROM FEATHER MEAL TO RYEGRASS STRAW ON RUMEN FERMENTATION *IN VITRO*

Treatment	CP ¹	GE ²	SUPPEN ³	IVDMD ⁴	IVNH3 ⁵	TOTVFA ⁶	C2 ⁷	C3 ⁷	C4 ⁷	C5 ⁷	I ⁷	A/P ⁸
Neg Control Run 5	6.03	4.07		22.8 ^{a+e}	.059 ^{a+d}	443 ^{a+d}	58 ^{a+c}	23 ^{a+j}	13 ^{a+c}	2.3	2.3	2.52 ^{a+i}
FM5 1	7.21	4.06	2.4	24.6 ^{b+f}	.115 ^{a+i}	449 ^{a+d}	62 ^{b,c}	22 ^{a+i}	11 ^{a,b}	1.5	1.8	2.74 ^{c+k}
2	8.97	4.04	6.0	24.3 ^{a+f}	.129 ^{a+j}	445 ^{a+d}	71 ^c	21 ^{a,b}	10 ^{a,b}	1.3	2.0	3.39 ^k
3	12.19	4.02	12.6	31.3 ^{g+q}	.263 ^{h+p}	683 ^{c,d}	57 ^{a,b}	25 ^{b+p}	12 ^{a+c}	2.6	1.6	2.28 ^{a+h}
4	17.10	3.98	23.0	37.8 ^{s+z}	.501 ^u	653 ^{b+d}	59 ^{a+c}	26 ^{d+q}	10 ^{a,b}	1.5	1.3	2.23 ^{a+h}
FM10 1	7.14	4.06	2.5	25.1 ^{b+g}	.083 ^{a+f}	500 ^{a+d}	59 ^{a+c}	24 ^{a+m}	12 ^{a+c}	2.0	2.8	2.47 ^{a+i}
2	8.82	4.05	6.3	27.1 ^{b+j}	.105 ^{a+h}	451 ^{a+d}	61 ^{b,c}	24 ^{a+m}	10 ^{a,b}	1.4	2.0	2.54 ^{a+i}
3	11.80	4.02	13.2	31.0 ^{f+q}	.229 ^{e+n}	644 ^{b+d}	47 ^a	23 ^{a+j}	12 ^{a+c}	2.1	1.9	1.99 ^{a+f}
4	16.44	3.99	24.0	39.1 ^{x+z}	.434 ^{s+u}	1024 ^e	58 ^{a+c}	26 ^{c+q}	11 ^{a,b}	1.8	1.6	2.24 ^{a+h}
Neg Control Run 6	6.03	4.07		20.2 ^a	.098 ^{a+h}	264 ^{a+c}	57 ^{a,b}	22 ^{a+h}	14 ^c	2.8	2.6	2.56 ^{a+i}
FM15 1	7.23	4.06	2.8	25.8 ^{b+i}	.108 ^{a+i}	234 ^{a+c}	51 ^{a,b}	21 ^{a+e}	16 ^c	6.8	3.9	2.35 ^{a+i}
2	8.74	4.06	6.5	27.7 ^{b+i}	.153 ^{a+k}	418 ^{a+d}	56 ^{a,b}	24 ^{a+n}	12 ^{a+c}	2.6	2.9	2.28 ^{a+h}
3	11.66	4.04	13.6	30.8 ^{f+q}	.245 ^{g+o}	416 ^{a+d}	57 ^{a,b}	25 ^{b+q}	13 ^{a+c}	2.0	1.6	2.21 ^{a+h}
4	16.15	4.02	24.6	35.7 ^{o+y}	.367 ^{n+u}	517 ^{a+d}	56 ^{a,b}	28 ^{k+r}	12 ^{a+c}	1.9	1.3	1.97 ^{a+e}
FM20 1	7.05	4.06	2.5	24.2 ^{a+f}	.117 ^{a+j}	508 ^{a+d}	58 ^{a+c}	24 ^{a+n}	13 ^{a+c}	1.8	2.3	2.33 ^{a+i}
2	9.05	4.04	7.7	28.4 ^{c+m}	.160 ^{a+k}	368 ^{a+d}	58 ^{a+c}	24 ^{a+i}	13 ^{a+c}	2.3	1.8	2.42 ^{a+i}
3	11.69	4.02	14.5	31.8 ^{h+s}	.198 ^{a+m}	457 ^{a+d}	63 ^{a+c}	23 ^{a+k}	10 ^{a,b}	1.1	1.6	2.66 ^{a+j}
4	15.75	3.99	25.1	36.1 ^{p+y}	.296 ^{l+s}	548 ^{a+d}	57 ^{a,b}	27 ^{f+q}	12 ^{a+c}	1.7	1.3	2.12 ^{a+g}
FM5LF5 1	6.83	4.05	2.1	26.1 ^{b+i}	.124 ^{a+j}	377 ^{a+d}	58 ^{a+c}	25 ^{b+o}	14 ^c	1.7	1.5	2.33 ^{a+i}
2	8.14	4.02	5.5	27.8 ^{b+m}	.171 ^{a+l}	381 ^{a+d}	58 ^{a+c}	24 ^{a+n}	12 ^{a+c}	1.9	2.1	2.36 ^{a+i}
3	10.35	3.96	11.6	31.1 ^{f+q}	.255 ^{h+p}	340 ^{a+d}	59 ^{a+c}	24 ^{a+n}	13 ^{b,c}	1.7	.9	2.42 ^{a+i}
4	13.98	3.87	21.8	38.4 ^{v+z}	.435 ^{t+u}	492 ^{a+d}	58 ^{a+c}	26 ^{d+q}	12 ^{a+c}	1.6	1.2	2.17 ^{a+li}

abcdefghijklmnopqrstuvwxyz Means in the same column with the same superscript across tables 5 to 8 are not different (P<.05)

¹CP = Crude protein, %

²GE = Gross energy, Mcal/kg

³SUPPEN = GE from supplement, %

⁴IVDMD = *In vitro* dry matter disappearance, %

⁵IVNH3 = *In vitro* ammonia production, mg/ml

⁶TOTVFA = Total volatile fatty acid production, μ mol/ml

⁷C2,C3,C4,C5,I = Acetate, propionate, butyrate, valerate and isomers of butyrate and valerate, respectively, % moles

⁸A/P = C2:C3 ratio

TABLE 8. EFFECT OF ADDED CP FROM LIQUEFIED FISH, UREA OR LIQUEFIED GRAIN STARCH TO RYEGRASS STRAW ON RUMEN FERMENTATION *IN VITRO*

Treatment	CP ¹	GE ²	SUPPEN ³	IVDMD ⁴	IVNH ₃ ⁵	TOTVFA ⁶	C2 ⁷	C3 ⁷	C4 ⁷	C5 ⁷	I ⁷	A/P ⁸
Neg Control Run 7	6.03	4.07		21.9 ^{a+b}	.034 ^a	362 ^{a+d}	59 ^{a+c}	24 ^{a+m}	10 ^{a+b}	1.8	3.4	2.44 ^{a+1}
LF5 1	7.35	4.06	3.1	22.4 ^{a+b}	.043 ^{a+b}	337 ^{a+d}	62 ^{b,c}	22 ^{a+g}	11 ^{a+c}	1.6	1.3	2.83 ^{a+k}
2	8.90	4.05	6.8	26.4 ^{b+j}	.088 ^{a+f}	454 ^{a+d}	59 ^{a+c}	24 ^{a+h}	12 ^{a+c}	1.9	1.6	2.44 ^{a+1}
3	11.66	4.03	13.4	32.4 ^{i+v}	.216 ^{c+m}	329 ^{a+d}	59 ^{a+c}	24 ^{a+n}	12 ^{a+c}	1.7	1.9	2.37 ^{a+1}
4	16.29	4.00	24.8	37.0 ^{q+z}	.411 ^{q+u}	573 ^{a+d}	56 ^{a,b}	26 ^{d+q}	11 ^{a+c}	2.5	1.8	2.11 ^{a+b}
LF10 1	6.97	4.07	2.1	18.9 ^a	.038 ^a	327 ^{a+d}	63 ^{b,c}	20 ^a	11 ^{a,b}	2.0	3.1	3.17 ^{i+k}
2	8.61	4.06	5.8	28.2 ^{c+m}	.057 ^{a+d}	557 ^{a+d}	59 ^{a+c}	25 ^{b+p}	10 ^{a,b}	1.7	1.6	2.34 ^{a+1}
3	11.58	4.05	12.5	29.5 ^{d+o}	.161 ^{a+1}	522 ^{a+d}	60 ^{a+c}	23 ^{a+k}	12 ^{a+c}	1.9	1.7	2.53 ^{a+1}
4	16.02	4.04	22.6	36.7 ^{p+y}	.398 ^{q+u}	640 ^{a+d}	57 ^{a,b}	28 ^{j+r}	10 ^{a,b}	1.8	1.6	2.04 ^{a+f}
LF15 1	6.92	4.07	1.8	22.6 ^{a+c}	.045 ^{a+c}	517 ^{a+d}	61 ^{b,c}	23 ^{a+1}	10 ^{a,b}	1.5	2.9	2.56 ^{a+1}
2	8.57	4.06	5.1	26.1 ^{b+1}	.067 ^{a+d}	446 ^{a+d}	59 ^{a+c}	24 ^{a+n}	11 ^{a,b}	2.5	2.1	2.40 ^{a+1}
3	11.43	4.05	10.9	27.9 ^{b+1}	.235 ^{f+n}	569 ^{a+d}	59 ^{a+c}	24 ^{a+1}	13 ^{a+c}	2.0	1.7	2.45 ^{a+1}
4	16.03	4.04	20.3	35.3 ^{n+x}	.415 ^{f+u}	606 ^{a+d}	57 ^{a,b}	25 ^{b+q}	12 ^{a+c}	2.4	1.9	2.21 ^{a+h}
LF20 1	6.98	4.08	1.9	22.7 ^{a+d}	.080 ^{a+f}	525 ^{a+d}	56 ^{a,b}	24 ^{a+1}	12 ^{a+c}	1.9	1.6	2.39 ^{a+1}
2	8.33	4.09	5.1	26.6 ^{b+j}	.100 ^{a+h}	673 ^{c,d}	60 ^{a+c}	24 ^{a+m}	12 ^{a+c}	1.5	1.2	2.49 ^{a+1}
3	11.31	4.12	11.5	28.5 ^{c+m}	.152 ^{a+k}	449 ^{a+d}	60 ^{a+c}	24 ^{a+n}	11 ^{b,c}	1.9	1.7	2.46 ^{a+1}
4	16.41	4.17	22.5	34.8 ^{n+x}	.385 ^{p+u}	774 ^d	55 ^{a,b}	26 ^{c+q}	12 ^{a+c}	2.9	2.7	2.11 ^{a+b}
UREA 1	7.04	4.05	1.5	21.3 ^a	.069 ^{a+e}	517 ^{a+d}	60 ^{a+c}	22 ^{a+1}	12 ^{a+c}	2.4	1.8	2.68 ^{a+j}
2	8.87	4.01	4.3	26.8 ^{b+j}	.128 ^{a+j}	551 ^{a+d}	57 ^{a,b}	25 ^{a+n}	12 ^{a+c}	2.3	1.9	2.31 ^{a+h}
3	12.13	3.94	9.5	28.0 ^{c+1}	.149 ^{a+1}	572 ^{a+d}	61 ^{b,c}	24 ^{a+m}	11 ^{a,b}	1.4	1.5	2.53 ^{a+1}
4	17.45	3.82	18.4	37.6 ^{a+z}	.464 ^{t,u}	546 ^{a+d}	59 ^{a+c}	27 ^{g+q}	11 ^{a,b}	1.4	.8	2.18 ^{a+h}
LGS 1	7.02	4.06	1.6	24.4 ^{a+f}	.280 ^{j+q}	469 ^{a+d}	60 ^{a+c}	21 ^{a+d}	13 ^{a+c}	2.7	2.0	2.79 ^{d+k}
2	9.07	4.06	4.9	28.5 ^{c+m}	.079 ^{a+f}	470 ^{a+d}	60 ^{a+c}	24 ^{a+n}	11 ^{a,b}	1.8	2.0	2.44 ^{a+1}
3	12.90	4.04	10.9	30.6 ^{f+p}	.206 ^{b+m}	584 ^{a+d}	63 ^{b,c}	23 ^{a+1}	10 ^{a,b}	1.4	1.3	2.76 ^{c+k}
4	19.54	4.01	20.8	33.4 ^{k+y}	.428 ^{g+u}	411 ^{a+d}	61 ^{b,c}	22 ^{a+h}	11 ^{a,b}	2.5	2.5	2.75 ^{b+k}

abcdefghijklmnopqrstuvwxy

Means in the same column with the same superscript across tables 5 to 8 are not different (P<.05).

¹CP = Crude protein, %

²GE = Gross energy, Mcal/kg

³SUPPEN = GE from supplement, %

⁴IVDMD = *In vitro* dry matter disappearance, %

⁵IVNH₃ = *In vitro* ammonia production, mg/ml

⁶TOTVFA = Total volatile fatty acid production, µmol/ml

⁷C2,C3,C4,C5,I = Acetate, propionate, butyrate, valerate and isomers of butyrate and valerate, respectively, % moles

⁸A/P = C2:C3 ratio

ratio than ranged from 1.73 to 3.39 for the 16.62 and 8.97 CP levels for HM20 and FM5, respectively ($P < .05$).

Regression analysis was used to predict IVDM from the nutritional components of the substrate. The parameters used to construct the equations were percent CP, percent CP², GE, GE², percent energy in the substrate from liquid supplement (SuppEN) and SuppEN². The equations constructed are shown in table 9 with r² values varying from .66 to .97 for SCP15 and LF10, and HM20 and FM10, respectively.

There was a significant response in consumption due to the different supplemental nitrogen sources as reported above. Therefore, stepwise regression analysis was also used in an attempt to predict consumption based on the nutrient composition of the diet (table 10). The amount of energy supplied by the supplement (as a percentage of total GE) was one of the principal factors controlling intake in the palatability trial, probably because of the higher digestible energy content of the liquid supplements. Straw and total DM (TotDM) consumption did not show a definite pattern since increases were found ($P < .05$) only when FM and LF were the supplemental nitrogen sources. Even then the equations constructed only accounted for 23 and 27%, respectively, of the variation in TotDM intake when FM and LF supplements were offered. Overall supplement consumption could be predicted with 65% and straw consumption with only 25% accuracy. The best estimate for TotDM consumption was the overall mean.

TABLE 9. REGRESSION EQUATIONS OF IVDMD ON THE PERCENTAGES OF NUTRIENT COMPOSITION OF THE SUBSTRATES.

Treatment	Regression Equation	R ²	S _{y.x} ^d
SCP5	IVDMD = -13.2 + 7.17 CP ^a - .23 CP ²	.90	1.00
SCP10	IVDMD = 18.94 + .29 CPEN ^b	.93	1.28
SCP15	IVDMD = 15.54 + 1.49 CP	.466	4.35
SCP20	IVDMD = 19.14 + .30 CPEN	.92	1.34
SCP5LF5	IVDMD = 21.56 + .068 CP ²	.95	1.40
HM5	IVDMD = 22.20 + .057 CP ²	.89	1.88
HM10	IVDMD = 15.56 + 1.41 CP	.96	1.17
HM15	IVDMD = 15.97 + .36 CPEN	.93	1.30
HM20	IVDMD = 24.82 + .56 SUPPEN ^c	.97	1.06
HM5LF5	IVDMD = 24.31 + .60 SUPPEN	.92	1.53
FM5	IVDMD = 20.94 + .06 CP ²	.93	1.60
FM10	IVDMD = 22.98 + .66 SUPPEN	.97	1.00
FM15	IVDMD = 3.16 + 3.69 CP - .104 CP ²	.94	1.47
FM20	IVDMD = 12.85 + 1.55 CP	.90	1.95
FM5LF5	IVDMD = 22.66 + .74 SUPPEN	.92	1.84
LF5	IVDMD = 21.75 + .65 SUPPEN	.92	1.81
LF10	IVDMD = 11.2 + 1.61 CP	.66	4.48
LF15	IVDMD = 13.88 + 1.32 CP	.85	2.16
LF20	IVDMD = 21.36 + .86 SUPPEN	.88	1.98
UREA	IVDMD = 21.36 + .86 SUPPEN	.88	2.23
LGS	IVDMD = 22.51 + 1.16 SUPPEN - .03 SUPPEN ²	.87	1.74

^a Crude protein, %

^b CP x gross energy, % and Mcal/kg, respectively.

^c Gross energy from supplement, %

^d Error mean square^{.5}, n = 15.

TABLE 10. REGRESSION EQUATIONS OF SUPPLEMENT, RYEGRASS STRAW AND TOTAL DRY MATTER CONSUMPTIONS ON NUTRIENT COMPOSITION OF THE DIET.

Treatment	Regression equation	R ²	S _{y.x} ^h
SCP	Supp ^a = -.59 + .17 SUPPEN ^d	.63	.67
HM	Supp = 1.95 + .0024 SUPPEN ²	.70	.39
FM	Supp = -.987 + .18 SUPPCP ^e	.65	.90
	TOTDM ^b = 5.91 + .15 LEVEL ^f	.23	1.64
LF	Straw ^c = 10.65 - .3 CP ^g	.55	1.09
	TOTDM = 9.60 - .0053 CP ²	.27	1.66
OVERALL	Supp = 1.61 + .28 SUPPEN - .0026 SUPPEN ²	.65	.81
	Straw = 6.15 - .00095 SUPPEN ²	.25	1.07

^a Supplement consumption, as fed, kg/d.

^b Total dry matter consumption, kg/d.

^c Straw consumption, DM, kg/d.

^d Gross energy from supplement, %.

^e Crude protein from supplement, %.

^f Percentage of feather meal in molasses.

^g Diet crude protein, %.

^h Error mean square^{.5}, n = 25 for all equations except for overall equations where n = 100.

DISCUSSION

A delicate balance between nitrogen and energy needs to be maintained to sustain a healthy ruminal microbial population, which in turn has a direct effect on voluntary intake. It is apparent that the most limiting factor is the dietary protein level, but it is now certain that NH_3 concentration in the rumen environment is a key factor (Church, 1979). Satter and Roffler (1977) reported that NH_3 concentrations above 5 mg/dl of rumen liquor are not utilized by rumen microflora for protein synthesis by cattle. In an earlier report (Allen and Miller, 1972), it was found that optimum nitrogen flow was attained at a concentration of 23.9 mg nitrogen/dl of rumen liquor with sheep receiving a high urea diet. The addition of molasses to a high urea corn-based diet has been shown to improve nutrient utilization and performance (Lawrence et al., 1974) as did the addition of preformed protein to a urea-molasses based liquid supplement (McMeniman et al., 1974) or diets with poor quality roughages (Jones et al., 1976; Kellems, 1980).

The results of the palatability trial reported here are difficult to compare to values in the literature since most of the experimental work has been done under restricted feeding or lower nutritional regimens. Supplemental consumption was greater in this trial than consumptions reported by Kellems (1980) with straw:liquid supplement ratios of 7.11 and 4 when urea was the sole supplemental nitrogen source or SCP replaced 10% of the CP in 30% CP liquid supplements. Kellems also reported ratios (straw/liquid supplement) of 6.1, 9.3, and 3.7 for urea treatments or when SCP or FM replaced 10% of the supplemental nitrogen, respectively.

When Kellems fed supplements with 10% of the supplemental nitrogen from LF, he observed ratios of 1.9. These results compare with ratios of 2.81, 2.35, 1.56 and 2.31, respectively, obtained in this study when 5 to 20% DM from SCP, HM, FM or LF were suspended in molasses, or 2.22 when urea was the sole supplemental nitrogen source.

The voluntary intake attained in this trial was greater than the intakes of DM of 3.08% BW obtained with buffaloes with molasses-urea and fish meal (Pathak et al., 1976), or 4.12 and 4.68 g/kg BW^{.75} observed by Hennessy et al. (1978) when 112 g of urea were infused with 500 or 1000 g of molasses to Hereford cattle. Crude protein consumption was lower than in another report from this station where yearling heifers consumed 4.18, 8.58 and 9.21 g CP/kg BW^{.75} with ryegrass straw and molasses-urea supplements with 7.5, 15 and 30% CP, respectively (Ortega et al., 1980).

Results from in vitro trials are said to permit a good estimate of in vivo digestion studies with correlation coefficients of 90% or better (Church, 1975). Recently, Jones et al. (1980) reported that proximate analysis components are more effective as a predictor of intake and digestibility of roughages by dairy cattle. The results reported here are compatible with the IVDMD obtained by Ortega et al. (1980) when ryegrass straw was incubated with liquid supplements varying in CP from 20 to 70%. In the later trial (Ortega et al., 1980), IVDMD increased as the ratio of CP to energy increased in isocaloric and isonitrogenous trials with maximum values of 42.10 and 46.02%, respectively.

Geerken (1978) reported a butyrate pattern of fermentation in vivo when molasses, hay and fish meal were fed to a sheep (43, 16 and 39% mol for acetate, propionate and butyrate, respectively). Urea-sulfur-molasses and urea-sulfur mixtures were compared to softwood-hardwood blend ammonium sulfite liquor in regards to in vitro VFA production (Kromann, 1976). The ammonium-sulfite liquor treatments increased TOTVFA, but propionic acid production was still higher with the molasses mixtures. In the present trial, propionic acid production was high and increased as the amount of supplement increased in the substrate; this was reflected by the C2:C3 ratio. The addition of DM from the different nitrogen sources did not have an effect on the pattern of fermentation within each liquid supplement. This pattern of fermentation is not surprising if we know that as the amount of readily available carbohydrates of the diet increases, propionic acid production increases while acetic and butyric tend to decrease (Church, 1975).

The results of the in vitro trials were also very poor estimators of DM intake in this study. Walters (1971) reported an equation ($Y=1.62X-30.8$; Y =intake, X =IVDMD) with r^2 of .69. The literature on this topic is rather extensive (Menke et al., 1979; Lamb and Eadie, 1979; Ortega et al., 1980), but frequently the equations reported are confusing. For example, Lamb and Eadie (1979) reported an equation relating the change in intake of roughage when barley was included in the diet ($r^2=.88$), but what is the level of intake of roughage alone?

CONCLUSION

In the present trial none of the supplemental nitrogen sources

were as effective in controlling voluntary intake of supplements or maximizing roughage consumption as supplements where urea and ammonium polyphosphate furnished most of the nitrogen in the supplements. This is reflected by the straw:liquid supplement ratios reported. On the other hand, the addition of the compounds under study were effective in improving IVDM which in turn should have an effect on in vivo performance due to a higher increase in propionic acid production. Further more critical experimental work needs to be done on this topic.

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CHAPTER II

Effect of different nitrogen sources on molasses-based liquid supplements for cattle. 2. In vivo evaluation¹.

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Key Words: Straw, in vivo digestibility, beef cattle, voluntary intake.

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SUMMARY

Fifteen crossbred Hereford heifers were used in in vivo digestion studies to quantify the effect of additional crude protein (0, 1, 2, 3 and 4 g/kg BW^{.75}) on voluntary intake and apparent digestibility of the nutritional components of the diet. The basal ration used was ryegrass straw while the crude protein sources were molasses-based liquid supplements where 10% of the supplemental nitrogen was replaced by either feather meal or liquefied fish (trial 1), or hair meal (trial 2). The feather meal liquid supplement promoted the highest consumption of straw, dry matter, crude protein and digestible energy (kg or g/kg BW^{.75}, and Mcal or Kcal/kg BW^{.75}, respectively; P<.05). Straw consumption was increased by the addition of 2 g crude protein/kg BW^{.75} with the feather meal and liquefied fish liquid supplements (P<.01), and total dry matter and crude protein consumptions were improved by the addition of 1 g crude protein/kg BW^{.75} from the three liquid supplements under study (P<.01). Digestible energy consumption was improved at the third and first level of crude protein supplementation (trial 1 and 2, P<.01 and P<.05; respectively). Nevertheless, the experimental animals never achieved a positive energy balance. Concentration of urea in blood plasma increased at the highest level of nitrogen supplementation (P<.01 and P<.05; trials 1 and 2, respectively). Total ruminal fatty acid production increased at the second and first level of supplemental crude protein for trial 1 and 2, respectively (P<.05 and P<.01) and changed to a propionate pattern of fermentation as diet protein was increased. This change decreased the acetate:propionate ratio (P<.01 and P<.05; trial 1 and 2, respectively). The liquefied fish liquid supplement had a detrimental effect on dry

matter and gross energy digestibilities ($P < .05$), while the feather and hair meal improved them ($P < .05$ and N. S., respectively). Crude protein digestibility was highly improved at the first level of crude protein supplementation with the three liquid supplements under study ($P < .01$). Crude protein supplementation ($\text{g/kg BW}^{.75}$) was the parameter that showed the highest correlation coefficients with consumption and digestibility, and was also the most important factor to predict consumption under the experimental conditions of this work.

INTRODUCTION

Capacity of the rumen, rate of passage and dry matter (DM) digestibility are the limiting factors regulating intake of low quality roughages (Baile and Forbes, 1974; Church, 1979). Nitrogen deficiency has been suggested as being the most important factor limiting digestible energy intake (Lyons *et al.*, 1970). When highly digestible roughages are offered, production requirements and endocrinological factors affecting appetite are key controllers of feed intake (Oh *et al.*, 1969; Baile and Forbes, 1974). Protein supplementation has been shown to improve utilization of poor quality roughages (Smith, 1962; Santos, 1979). An increase in intake of 42% was noted when casein (4.5 g nitrogen/d) was infused in the duodenum of sheep with a 3.5% CP chaffed oaten hay (Smith, 1962). The voluntary intake of poor quality roughages (grass hay or wheat straw) was increased by the addition of 1 g CP/kg $\text{BW}^{.75}$ from soybean or cottonseed meal. However, no improvement in intake was noted when the protein source was urea in a molasses-based liquid supplement (Santos, 1979). Addition of readily available carbohydrates is necessary before urea has

an effect upon roughage intake. Maximum intake and rate of passage occurred when urea (6-16 g/d) was administered to sheep on straw and 3% molasses diets (Coombe and Tribe, 1963). In metabolic studies with sheep, the use of liquid supplements with whey or fababean starch improved dry matter digestibility and nitrogen retention over urea alone on a high fiber diet (Steeds et al., 1979).

The present trials were conducted to study the possibility of improving ryegrass straw utilization with liquid supplements. Protein sources (besides urea) were liquefied fish, hair meal or feather meal in molasses-urea supplements.

MATERIALS AND METHODS

Three molasses-urea based liquid supplements (30% CP) were made where 10% of the nitrogen was supplied by hair meal (HM), feather meal (FM; 5-03-795) or liquefied fish from whole hake (LF; Law, 1973; table 1). Trial 1. The FM and LF liquid supplements were fed in fixed amounts of 0, 1, 2, 3 and 4 g CP/kg of metabolic body weight ($BW^{.75}$) to study the response to the added nitrogen from the liquid supplements in regards to DM intake and digestibility of DM, CP, acid detergent fiber (ADF), and energy. Ten crossbred yearling heifers were used for these purposes. The animals were maintained on ryegrass straw (1-04-059), water and trace mineralized salt free choice. Straw consumption was monitored for each animal daily. The experimental animals were maintained on each treatment for two weeks. Fecal samples were collected for three consecutive days at the end of each treatment period. Fecal and feed samples were analyzed for CP, DM, ADF, lignin and gross energy (AOAC, 1975). Rumen fluid and

TABLE 1. COMPOSITION OF LIQUID SUPPLEMENTS AND RYEGRASS STRAW

Composition, %	Liquid Supplements			Ryegrass Straw	
	FM	HM	LF	Trial 1	Trial 2
Urea	8.16	8.16	8.21		
Molasses	68.96	68.96	68.82		
Water	16.33	16.33	-		
Feather meal	3.22	-	-		
Hair meal	-	3.22	-		
Liquefied fish	-	-	19.90		
Sulfur	.21	.21	.21		
Phosphoric acid (83%)	2.00	2.00	1.74		
Trace mineral salt	.97	.97	.97		
Kelflo ^a	.15	.15	.15		
Analytical data					
Dry matter, %	69.22	64.06	64.26	92.43	91.69
Crude protein, %	29.28	29.39	29.12	6.36	3.89
Gross energy, Mcal/kg	2.21	2.06	2.16	4.00	3.81
Acid detergent fiber, %	2.24	3.40	4.75	49.48	48.71
Lignin, %	1.55	2.73	3.78	8.49	7.82

^aKelko, Division of Merck & Co., Inc.

blood samples were also collected at the end of each treatment period for blood urea nitrogen (BUN) and volatile fatty acids (VFA) determinations. Blood samples were centrifuged at 1200 rpm for 15 min. and plasma was frozen for later BUN determination¹. Rumen liquor samples were prepared for VFA determination by a method similar to that described by Baumgardt (1964), and the analysis conducted on a gas liquid chromatograph².

Apparent digestibility of feed components was calculated by the indicator method using lignin as internal indicator (Schneider and Flatt, 1975). A completely randomized design was used for the analysis of variance of the results and multiple regression analyses were conducted to show dependency of voluntary intake upon diet protein (Neter and Wasserman, 1974; Gill, 1978).

Trial 2. This experiment was similar to trial 1, except that the liquid supplement used has HM as the supplemental nitrogen source (HM) and the amount of liquid supplement fed to five crossbred Hereford heifers was 0, 1 and 3 g CP/kg BW^{.75}. The remainder of the experimental procedure was the same as that for trial 1.

RESULTS

Trial 1. Consumptions of straw, DM, DE and CP per unit of weight or metabolic body weight were higher when the supplemental nitrogen source

¹Pierce Urea Nitrogen Rapid Statkit, Pierce Chemical Co., Rockford, IL 61105.

²Varian-Aerograph (model 1200). Varian-Aerograph Co., Walnut Creek, CA

(besides urea) in the liquid supplement was FM (table 2; $P < .05$). The addition of CP to the straw basal ration had an effect on consumption of the different parameters measured (table 3; $P < .01$). Straw consumption (kg DM) increased when 2 g CP/kg $BW^{.75}$ were added to the diet and then leveled off. The same tendency was observed in regards to straw consumption per unit of $BW^{.75}$, but in the last case the highest CP level (4 g supplemental CP/kg $BW^{.75}$) resulted in a greater consumption than level one (1 g supplemental CP/kg $BW^{.75}$; $P < .01$). Total DE consumption and DE consumption per kg $BW^{.75}$ did not increase until 3 g supplemental CP/kg $BW^{.75}$ were added to the diet ($P < .01$). Increased total DM consumption was noted at the first and second levels of protein supplementation with no further increase ($P < .01$), while DM consumption per kg $BW^{.75}$ did not increase until 2 g CP/kg $BW^{.75}$ were added to the diet. Total CP consumption and CP consumption/kg $BW^{.75}$ increased as the level of diet protein was increased.

Table 4 shows the results of ruminal VFA production and BUN determinations ($\mu\text{mol/dl}$, percentage molar and mg/dl). The amount of urea in blood was higher for the highest level of diet CP as compared to the 0 level of protein supplementation, but this mean was not different from level one ($P > .01$). Highest total VFA production was attained at the second level of CP supplementation and this average was greater than VFA production in level 3 ($P < .05$). Acetic acid (C2; % molar) was higher with the basal straw ration than for the 2 and 4 CP supplementation levels ($P < .01$). The inverse tendency was noted for propionic and butyric acids (C3 and C4; % molar; $P < .01$). There was no definite trend for changes in valeric, isovaleric or isobutyric acid productions (OTHERS;

TABLE 2. CONSUMPTIONS ACHIEVED BY EXPERIMENTAL ANIMALS WHEN THE SUPPLEMENTAL N SOURCES (BESIDES UREA) WERE FEATHER MEAL (FM) OR LIQUEFIED FISH (LF)

Consumption	Supplemental N Source Besides Urea	
	FM	LF
Ryegrass straw DM, kg	5.0 ^a	4.8
Ryegrass straw DM, g/kg BW ^{.75}	64.8 ^a	60.6
Total DM, kg	5.5 ^a	5.2
Total DM, g/kg BW ^{.75}	69.1 ^a	65.0
CP, kg	.5 ^a	.5
CP, g/kg BW ^{.75}	6.5 ^a	6.2
DE, Mcal	9.1 ^a	8.2
DE, Kcal/kg BW ^{.75}	114.4 ^a	103.0

^aMeans within the same row with different superscript are different ($P < .05$).

TABLE 3. CONSUMPTIONS ACHIEVED BY EXPERIMENTAL ANIMALS AT THE DIFFERENT CRUDE PROTEIN SUPPLEMENTATION LEVELS IN TRIAL 1

Consumption	Level of Supplemental Crude Protein (g/kg BW ^{.75})				
	0	1	2	3	4
Ryegrass straw DM, kg	4.3 ^a	4.7 ^{ab}	5.1 ^b	5.1 ^b	5.4 ^b
Ryegrass straw DM, g/kg BW ^{.75}	54.7 ^a	59.5 ^{ab}	65.2 ^{bc}	64.8 ^{bc}	69.2 ^c
Total DM, kg	4.3 ^a	4.9 ^b	5.5 ^c	5.6 ^c	6.1 ^c
Total DM, g/kg BW ^{.75}	54.7 ^a	61.6 ^a	69.5 ^b	71.4 ^b	77.8 ^b
CP, kg	.3 ^a	.4 ^b	.5 ^c	.6 ^d	.6
CP, g/kg BW ^{.75}	3.7 ^a	5.0 ^b	6.4 ^c	7.4 ^d	8.7
DE, Mcal	7.2 ^a	8.1 ^{ab}	8.4 ^{ab}	9.4 ^{bc}	10.0 ^c
DE, Kcal/kg BW ^{.75}	91.0 ^a	102.2 ^{ab}	105.3 ^{ab}	118.1 ^{bc}	126.7 ^c

^{abc} Means in the same column with the same superscript are not different (P>.01).

TABLE 4. BLOOD UREA N LEVELS (BUN) AND VOLATILE FATTY ACID PRODUCTIONS OBSERVED IN THE EXPERIMENTAL ANIMALS IN TRIAL 1

Item	Level of Supplemental Crude Protein (g/kg BW ^{.75})				
	0	1	2	3	4
BUN, mg/dl	11.4 ^c	18.7 ^{cd}	19.3 ^{cd}	24.4 ^{cd}	31.1 ^d
Tot VFA ^f , μ mol/dl	367.7 ^{ab}	385.2 ^{ab}	413.7 ^b	347.5 ^a	391.3 ^{ab}
C2 ^g , % molar	68.4 ^d	65.2 ^{cd}	60.1 ^c	64.7 ^{cd}	61.4 ^c
C3 ^g , % molar	19.7 ^c	21.0 ^{cd}	22.5 ^d	21.2 ^{cd}	22.5 ^d
C4 ^g , % molar	8.5 ^c	9.9 ^c	11.7 ^d	11.3 ^{cd}	12.5 ^d
Others ^e , % molar	3.4	4.0	5.8	2.7	3.6
C2/C3	3.6 ^d	3.1 ^{cd}	2.7 ^c	3.1 ^{cd}	2.7 ^c

^{a,b} Means in the same row with the same superscript are not different (P>.05).

^{c,d} Means in the same row with the same superscript are not different (P>.01).

^e Isobutyric and valeric and isovaleric acids.

^f Total volatile fatty acid

^g C2 = acetate, C3 = propionate, C4 = butyrate

table 4). The ratio of C2:C3 decreased with the increments in diet protein as was expected from the production of the two acids by themselves ($P < .01$).

Digestibility of DM and GE were negatively affected by the addition of CP to the diet when LF was the supplemental nitrogen source (besides urea) in the liquid supplements, while an improvement was noted when FM was added (table 5; $P < .05$). Higher DM digestibility values were obtained with the 3 and 4 g supplemental CP/kg BW^{.75} levels with FM liquid supplement and for the basal ration (straw) in the LF treatment. The lower values were with the basal ration in the FM treatment and 2 g supplemental CP level in the LF treatment. The same tendency was followed for GE digestibility with a range of 34.8 to 41.4% (2 and 0 g supplemental CP/kg BW^{.75} for the LF treatments, respectively; $P < .05$). Apparent digestibility of CP (table 6) was increased with one and again with 4 g supplemental CP/kg BW^{.75} ($P < .01$), while the digestibility of ADF was affected negatively ($P < .01$).

Regression analysis was used in an attempt to predict straw, DM CP and DE consumptions (table 11). The independent variable being more important to predict intakes was the supplemental CP level/kg BW^{.75}. The parameter that was predicted with highest accuracy was CP consumption/kg BW^{.75} with r^2 values of .944 and .930 for the FM and LF treatments, respectively. The correlation coefficients between straw DM, CP and DE consumptions (kg, g/kg BW^{.75}, and Kcal/kg BW^{.75}), apparent digestion coefficients and dietary DE (Mcal/kg) are shown in table 10. Straw consumption (g DM/kg BW^{.75}) was highly correlated with DM, CP (g/kg BW^{.75}) and DE (Kcal/kg BW^{.75}) consumptions ($P < .01$). Total DM consumption

TABLE 5. APPARENT DIGESTION COEFFICIENTS FOR DRY MATTER AND GROSS ENERGY OBTAINED WITH THE FEATHER MEAL (FM) OR LIQUEFIED FISH (LF) LIQUID SUPPLEMENTS

Apparent Digestion Coefficient		Level of Supplemental Crude Protein (g/kg BW ^{.75})				
		0	1	2	3	4
Dry matter	FM	33.7 ^{ab}	38.1 ^{bc}	34.4 ^{abc}	38.8 ^c	38.7 ^c
	LF	39.0 ^c	34.5 ^{abc}	33.3 ^a	36.4 ^{abc}	34.8 ^{abc}
Gross energy	FM	35.7 ^{de}	40.0 ^{def}	36.3 ^{def}	41.4 ^{ef}	40.8 ^{ef}
	LF	41.4 ^f	37.3 ^{def}	34.8 ^d	37.3 ^{def}	36.2 ^{def}

a,b,c,d,e,f Means with the same superscript are not different (P>.05).

TABLE 6. APPARENT DIGESTION COEFFICIENTS FOR CRUDE PROTEIN AND ACID DETERGENT FIBER OBTAINED IN TRIAL 1

<u>Apparent Digestion Coefficient</u>	<u>Level of Supplemental Crude Protein (g/kg BW^{.75})</u>				
	0	1	2	3	4
Crude protein	32.1 ^a	45.5 ^b	47.7 ^b	50.7 ^b	55.7
Acid detergent fiber	31.7 ^b	28.9 ^{ab}	24.6 ^a	30.3 ^b	26.3 ^{ab}

^{a, b} Means with different superscript are not different (P>.01).

followed the same tendency except that this parameter was also related to CP digestibility (FM and LF; $P < .05$ and $P < .01$, respectively) and ADF digestibility (LF; $P < .05$). DE content of the diet (Mcal/kg) was highly correlated with DE intake (Kcal/kg $BW^{.75}$) and the apparent digestion coefficients of the nutritional components of the diet.

Trial 2. No significant effect was noted in regards to straw consumption (DM; kg and g/kg $BW^{.75}$) for the HM liquid supplement (table 7). Total DM and DE consumptions (kg and g/kg $BW^{.75}$) increased with the increase of supplemental CP in the diet (1 g supplemental CP/kg $BW^{.75}$; $P < .05$) without any further increment. Total CP intake (kg and g/kg $BW^{.75}$) increased with each increment of dietetic CP ($P < .01$).

Table 8 shows the increment in BUN at the highest level of CP supplementation with the HM liquid supplement ($P < .05$). Total VFA production increased at the intermediary level of supplemental CP (1 g supplemental CP/kg $BW^{.75}$) without any further increment ($P < .01$). No effect due to treatment levels was found in regards to C2; but C3 and C4 increased with the first level of CP supplementation and then leveled off ($P < .05$). The ratio of C2:C3 decreased with the 3 g supplemental CP /kg $BW^{.75}$ ($P < .05$).

There was no effect due to the treatment levels in trial 2 in regards to digestibilities of the nutritional components of the diet except for CP where its digestion coefficients increased with the first increment in dietary CP (table 8; $P < .05$).

Along with the experimental animals of this trial, four Holstein steers and a Hereford heifer was also fed HM liquid supplement. The consumption achieved by the Holstein group was similar to the intakes

TABLE 7. CONSUMPTIONS ACHIEVED BY EXPERIMENTAL ANIMALS WHEN HAIR MEAL (HM) WAS THE SUPPLEMENTAL N SOURCE (BESIDES UREA) IN TRIAL 2

Consumption	Level of Supplemental Crude Protein (g/kg BW ^{.75})		
	0	1	3
Ryegrass straw DM, kg	3.3	3.9	3.9
Ryegrass straw DM, g/kg BW ^{.75}	49.2	58.3	57.3
Total DM, kg	3.3 ^a	4.1 ^b	4.3 ^b
Total DM, g/kg BW ^{.75}	49.2 ^a	60.5 ^b	63.8 ^b
CP, kg	.1 ^c	.2 ^d	.3
CP, g/kg BW ^{.75}	2.0 ^c	3.4 ^d	5.4
DE, Mcal	4.0 ^a	7.2 ^b	6.7 ^b
DE, Kcal/kg BW ^{.75}	58.3 ^a	106.6 ^b	98.0 ^b

^{a,b} Means in the same row with the same superscript are not different (P>.05).

^{c,d} Means in the same row with the same superscript are not different (P>.01).

TABLE 8. BLOOD UREA N (BUN) AND VOLATILE FATTY ACID PRODUCTIONS OBSERVED IN THE EXPERIMENTAL ANIMALS IN TRIAL 2.

Item	Levels of Supplemental Crude Protein (g/kg BW ^{.75})		
	0	1	3
BUN, mg/dl	10.5 ^a	9.4 ^a	22.1
Tot VFA ^f , μmol/dl	420.8 ^c	508.7 ^d	557.7 ^d
C2 ^g , % molar	62.6	62.7	60.0
C3 ^g , % molar	21.3 ^a	23.1 ^b	24.5 ^b
C4 ^g , % molar	9.6 ^a	11.0 ^b	11.3 ^b
Others ^e , % molar	6.5	3.2	4.3
C2/C3	3.0 ^b	2.7 ^b	2.5 ^a

^{a,b} Means in the same row with the same superscript are not different (P>.05)

^{c,d} Means in the same row with the same superscript are not different (P>.01)

^e Isobutyric and valeric and isovaleric acids.

^f Total volatile fatty acids

^g C2 = acetate, C3 = propionate, C4 = butyrate

TABLE 9. APPARENT DIGESTION COEFFICIENTS OBTAINED FOR DRY MATTER, CRUDE PROTEIN, GROSS ENERGY AND ACID DETERGENT FIBER IN TRIAL 2.

Apparent Digestion Coefficient	Level of Supplemental Crude Protein (g/kg BW ^{.75})		
	0	1	3
Dry matter	33.1	45.0	42.2
Crude protein	1.8 ^a	36.9 ^b	51.4 ^b
Gross energy	29.6	42.7	37.6
Acid detergent fiber	40.5	49.0	42.2

a,b Means with the same superscript are not different (P>.01).

of their counterparts (Hereford group). At the highest level of CP supplementation (4 g/kg BW^{.75}), the Holstein group showed a marked decrease in digestibility with average DM, CP, GE and ADF digestion coefficients of 17.6, 33.6, 10.7 and 15.7%, respectively.

The relationship found between consumption and digestibility was similar to trial one (table 10). The regression equations constructed to predict consumption when HM was the supplemental source of nitrogen (besides urea) are in table 11. No equation to predict straw consumption reached significance; therefore the best estimate was the mean.

DISCUSSION

The principal nitrogen sources used to make the liquid supplements was urea (about 90%) which is rapidly hydrolyzed to ammonia (NH₃) by bacterial urease. Provided sufficient digestible carbohydrate is available, rumen microbial populations can use NH₃ and carbohydrate for growth (Helmer and Bartley, 1971; Church, 1979). These factors have a direct effect in maintaining a healthy rumen microbial population which, in turn, affects intake and utilization of roughages. These assumptions hold if the dietary CP level is < 10% and if the ruminal NH₃ concentration remains < 5 mg/dl of rumen fluid (Elliot, 1967; Satter and Roffler, 1977). In the present report, roughage intake increased up to the 2 g supplemental CP/kg BW^{.75} level (equivalent to a protein content of 9.38%) without any further increase. In trial 2, the addition of liquid supplement failed to show this response even though the highest CP level achieved was only 8.51%.

In general, molasses-urea supplementation has failed to show

TABLE 10. CORRELATION COEFFICIENTS FOUND BETWEEN INTAKES AND DIGESTIBILITIES FOR THE FM, LF AND HM LIQUID SUPPLEMENTS

	DMMBW	DEMBW	CPMBW	DMD	CPD	ADFD	ENDIG	DIET DE ^e
<u>FM Liquid Supplement</u>								
Straw MBW ^a	.97 **	.82 **	.77 **	.03	.26 *	-.26	.00	-.02
DMMBW ^b		.87 **	.89 **	.12 **	.42 **	-.28	.10 **	-.06 **
DEMBW ^c			.86	.54 **	.62 **	.08	.56	.52
CPMBW ^b				.29	.70 **	-.21 **	.28 **	.22 **
DMD ^d					.63	.71 **	.93 **	.93 **
CPD ^d						.04	.62 **	.58 **
ADFD ^d							.68 **	.71 **
ENDIG ^d								.99
<u>LF Liquid Supplement</u>								
Straw MBW	.96 **	.66 **	.76 **	-.30	.470 *	-.42 *	-.34	-.36 *
DMMBW		.66 **	.90 **	-.31 *	.650 **	-.46 *	-.37 *	-.41 *
DEMBW			.57	.44 *	.552 **	.17 *	.43	.39
CPMBW				-.28	.843 **	-.46 **	-.36 **	-.43 **
DMD					-.027	.79 **	.94	.93
CPD						-.36	-.08 **	-.16 **
ADFD							.77	.78 **
ENDIG								.99
<u>HM Liquid Supplement</u>								
Straw MBW	.95 **	.67 **	.76 **	.17	.333	-.02	.17	.20 **
DMMBW		.67 **	.76 *	.17 **	.541 **	-.03 **	.22 **	.85 **
DEMBW			.55	.82 **	.788 **	.70 **	.86 **	.21 **
CPMBW				.29	.818 **	-.04 **	.25 **	.97 **
DMD					.730	.92 *	.97 **	.69 **
CPD						.47	.71 **	.69 **
ADFD							.93	.94 **
ENDIG								.99

^a g/kg BW^{.75}

^b g/kg BW^{.75}

^c Kcal/kg BW^{.75}

^d %

^e Mcal/kg

* P < .05

** P < .01

TABLE 11. REGRESSION EQUATIONS OF STRAW, DRY MATTER, CRUDE PROTEIN AND DIGESTIBLE ENERGY CONSUMPTIONS ON NUTRITIONAL COMPONENTS OF THE DIET WHEN HM, FM OR LF SUBSTITUTED 10% OF TOTAL CRUDE PROTEIN ON LIQUID SUPPLEMENTS .

Equation	R ²	S _{y.x} ^f
<u>Straw Consumption, kg</u>		
FM = 5.24 + .97 CPMBW ^a - .072 DP ^{2,b}	.60	.55
LF = 4.75 + .23 CPMBW	.18	.71
<u>Dry Matter Consumption, kg</u>		
FM = 4.84 + 1.05 CPMBW - .065 DP ²	.73	.51
LF = 4.39 + .38 CPMBW	.41	.69
HM = 3.45 + .00014 DP·DE ^c	.48	.42
<u>Dry Matter Consumption, g/kg BW^{.75}</u>		
FM = 62.40 + 15.09 CPMBW - 1.05 DP ²	.74	6.48
LF = 57.15 + 9.63 - .54 DP ²	.66	5.88
HM = 50.65 + .002 DP·DE	.40	7.16
<u>Crude Protein Consumption, g/kg BW^{.75}</u>		
FM = 4.29 + 1.89 CPMBW - .072 DP ²	.94	.48
LF = 3.75 + 1.20 CPMBW	.93	.48
HM = 2.22 + 1.09 CPMBW	.96	.31
<u>Digestible Energy Consumption, Kcal/kg BW^{.75}</u>		
FM = 37.23 + 25.93 CPMBW - 1.89 DP ² + .000024 DE ^{2,d}	.82	11.09
LF = - 26.05 + 13.15 CPMBW + .077 DE - .003 DP·DE	.63	10.15
HM = 45.18 + .0000075 DE ² - .0039 DADF ^e	.90	9.35

^a Crude protein supplementation, g/kg BW^{.75}

^b Digestible protein, percentage square.

^c Digestible protein and energy interaction, percentages.

^d Digestible energy, percentage square.

^e Digestible acid detergent fiber, percentage.

^f Error mean square^{.5}, n = 25

a response in poor quality roughage intake (Hennessy et al., 1978; Santos, 1979); although a positive response has been found with molassed sugar-beet pulp urea (Fishwick et al., 1973, 1974) or barley-urea (Fishwick et al., 1978). In the experiment reported by Santos (1979), he was feeding wheat straw with a CP content of 2.59% and his animals only achieved an intake of 51 g DM/kg BW^{.75} when 4 g supplemental CP/kg BW^{.75} in the form of molasses-urea were added to the diet. The increase in straw intake of 19.9% observed in the present trial is very close to the increase in intake of oat straw of 21% when molasses sugar-beet pulp urea were added to the diet of pregnant beef heifers (Fishwick et al., 1974).

Another important factor limiting digestibility and performance is the intake of energy. Attempts had been made to find the level of supplementary protein that will allow the animal to consume enough roughage to meet their energy requirements for maintenance (Elliot, 1967; Santos, 1979). According to Blaxter (1962), the suggested DE requirement for maintenance is 136 Kcal/kg BW^{.73}. This value is equivalent to 155 Kcal/kg BW^{.75}, and such an intake was never achieved in the present trials (tables 3 and 7).

The improvement in apparent digestibility of the nutritional components of the diet due to nitrogen supplementation is another factor that has been well documented (Schneider and Flatt, 1975; Church, 1979). The addition of urea to molasses has improved utilization of the nutritional components of the diet (mainly CP), but once enough urea has been added to the diet to bring the animal into small positive

nitrogen balance, additional urea is mostly excreted in the urine without promoting nitrogen deposition in the tissues (Coombe and Tribe, 1963; Ernst et al., 1975). In the present report the first level of CP supplementation to the diet had a significant positive effect on CP digestion coefficients without any further improvement (trial 2) or a second increase at the 4 g supplemental CP/kg BW^{.75} level (trial 1). These results are in agreement with the available literature. DM and GE digestibilities were initially improved, too, with the FM and HM supplements, but they declined with increased CP. The logical explanation for the decrease in fiber digestibility is the increase in intake with the subsequent increase in rate of passage. The detrimental effect of the LF liquid supplements on DM and GE digestibilities is difficult to explain and contrary to a recent report where the addition of LF to a molasses-urea supplement improved nutrient utilization by sheep (Shqueir et al., 1980).

Concentration of BUN is increased by increments in dietary protein concentration, reduction in intake or fasting (the first 2 or 3 days), or the addition of urea to low protein diets (Varady et al., 1970; Kirk and Walker, 1976). The BUN concentrations observed in the experiments reported here followed this pattern since there was a significant increase at the high levels of nitrogen supplementation and the values obtained were similar to those observed by Fishwick et al. (1973, 1978) when our experimental animals were maintained on straw alone.

Ruminal VFA production may be increased by the addition of molasses-urea supplements to the diet (Hennessy et al., 1978). The pattern of fermentation is also modified when readily available

carbohydrates are added with an increase in molar proportions of C3 (Fishwick et al., 1973, 1978; Hennessy et al., 1978; Church, 1979); that was the pattern found in these studies.

The relationship observed between the different parameters measured were similar to those reported by Santos (1979). It is interesting to observe that consumption was not related to the DM or ADF digestion coefficients as expected. Rather, there was a closer association with CP digestibility. This is no surprise if we realize that the CP digestion coefficient was the most greatly affected by the treatment levels and increased at the different levels of nitrogen supplementation. This was also shown by the highly significant association between CP intake and digestibility.

When intake was estimated by the use of regression analysis, the equations constructed followed the trend expected by the correlation coefficients shown in table 10. CP was the single most important factor to predict consumption, but, in most cases, complex equations were found to give better estimates of intake (more than 90% of the variability in CP consumption/kg BW^{.75}).

CONCLUSION

Data from this experiment show that the addition of preformed protein is beneficial to the use of molasses-urea based liquid supplements. Feather meal proved to be the best source of supplemental protein tested and it stimulated a greater intake of roughage. Hair meal improved the digestibility of the nutritional components of the diet to a higher degree, but this improvement was not significant. In the case of LF, the

results are contradictory to available literature and additional research will be required to determine its true effect.

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