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THE USE OF ALFALFA PROTEIN (PRO- XAN) IN MILK REPLACER DIEST FOR CALVES[1]

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Summary: *Alfalfa protein was reconstituted for milk protein in milk replacer formulations at five levels (0,25, 50, 75 and 100 %). One growth and one digestibility trial were performed at each level. Three Holstein male calves, 3 to 5 days of age were used in each trial. The calves were fed only liquid milk replacers for five weeks in digestibility trials. Total fecal and urine collections were made in the fourth week. In growth trials, in addition to the liquid diets, the calves received free of choice alfalfa hay and a calf starter. The calves were weaned in the fifth week and the experiments were terminated at the end of the eighth week.*

The average daily gains of the calves in the first five weeks period were: 220, 216, 187, 180 and 134 g for the digestion trials ($P < 0.05$) and 519, 450, 506, 329 and 377 g for the growth trials at 0,25, 50,75 and 100 % substitution levels, respectively. Post-weaning gains were similar for the trials and the levels.

Apparent dry matter, nitrogen and energy digestibilities decreased as the protein substitution levels were increased ($P < 0.01$). The average values of nitrogen retention decreased ($P < 0.05$) in the same direction. The mean nitrogen digestibility values were 85, 78, 77, 67 and 60 % in the above order. The association between substitution level and average daily gain is linear and the average daily gain decreased 1.99 g for every 1 % increase in AP substitution.

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Although alfalfa protein was less digestible than milk protein, the substitutions were well accepted by the calves. The results may be interpreted that alfalfa protein has a good potential as a substitute for milk protein in calf milk replacers.

Özet: *Buzağı İkame Sütlerinde Yonca Proteini Konsantresinin (Pro-Xan) Kullanılması.*

Çalışmada kullanılan yonca proteini konsantresi yeşil yoncadan elde edilmiştir. İkame süt formulasyonlarında yonca proteini, süt proteininin yerini % 0,25, 50, 75 ve 100 *düzeylerinde almıştır. Her düzeyde bir hazım bir de büyüme olmak üzere iki grup deneme yapılmıştır. Her denemede* 3–5 *günlük üçer baş Holştayn erkek buzağı kullanılmıştır. Araştırmanın sütle besleme dönemi beş, süt kesimi sonrası üç hafta sürmüştür. Hazım denemelerinde ilk* 5 *haftada buzağılara yalnız ikame süt verilmiş ve dördüncü hafta içinde buzağılar hazım bokslarına konularak* 6 *gün süre ile dışkı ve idrarları toplanmıştır.*

Hazım denemelerinde buzağıların ilk beş haftadaki günlük ağırlık artışları % 0, 25, 50, 75 *ve* 100 *düzeylerde, sırası ile,* 220, 216, 187, 180 ve 134 *g bulunmuş olup düzeyler arası farklar önemlidir* ($P < 0.05$). *Formulasyonda yonca proteininin artması ile hazmolabilir enerji, nitrojen ve kuru madde oranları azalmıştır* ($P < 0.01$). *Nitrojen retensiyonu da aynı yönde azalma göstermiştir* ($P < 0.05$).

Büyüme denemelerinin ilk beş haftalık döneminde düzeylerdeki ortalama günlük ağırlık artışı, yukarıki sıra ile, 519, 450, 506, 329 ve 377 *g olup düzeyler arası farklar önemsizdir. Buzağılarda ishalli günler sayısı, gaita yumuşaklığı, dehidrasyon, tavır ve genel durum için yapılan puvanlama ortalamaları arası farklar da önemsizdir. Süt kesimi sonrası için gerek hazım ve büyüme dönemleri, gerekse düzeyler arası farklar önemsizdir.*

Yonca proteini hem nitrojen hem de enerji hazmolabilirliği yönünden süt proteinine göre daha düşük değerdedir ve koagule bir protein olduğundan suda erime ve suspansiyon özelliği azdır. Bununla beraber buzağıların % 75 *düzeye kadar tatmin edici büyüme performansı göstermeleri yonca proteininin ikame süt formulasyonları için önemli bir protein kaynağı olabileceğini ifade etmektedir. Buzağılar bütün düzeylerdeki ikame sütleri severek içmişlerdir.*

Introduction

The most common, currently used protein source in calf milk replacers is powdered skim milk. However, the cost of milk protein is high and there is an increasing competition for it on the human food market. Since proteins from plant origin are more abundant and generally less expensive than those of animal origin it would

be advantageous if an appropriate plant protein could be used in milk replacer diets.

Although soybean protein is used in some commercial milk replacers, some limitations are still present (9). The use of single cell proteins in milk replacers hale also been reported, but the digestibility of sigle cell protein is low and it can only supply about 25 % of the total dietary protein (4).

Alfalfa has a very high potential as a protein source, however, the protein content must be separated from other leaf constituents if it is to be used in milk replacers for calves. Pirie (7) pioneered a processing method in England during World War II for the separation of leaf protein for human consumption. Recently, the researchers, at the USDA–Western Regional Research Center have developed an economically feasible method of producing alfalfa protein concentrate (APC) from green alfalfa (5). The product has been named Pro–Xan.

APC contains about 56 % of crude protein and its amino acid composition is more similar to that of skim milk than that of soy protein. It has been reported that APC gave favorable results in feeding young pigs and poultry (1, 6). A report, on the other hand, indicated that 50 % substitution of protein from alfalfa coagulum for milk protein in milk replacers resulted in decreases in average daily gains of calves (10). The objectives of this study were to investigate the possibility of replacing milk proteins with alfalfa proteins in milk replacers for dairy calves and to determine rates of gain, feed efficiency and survival abilities of calves on milk replacers containing different levels of alfalfa protein (AP).

Materials and Methods

Two types of investigations were conducted, concerned with calf growth and digestibility of APC, respectively. In each type of trial five levels of AP substitution (e.g. 0, 25, 50, 75 and 100 %) for milk protein were tested. The ingredients and chemical composition of the milk replacer diets are given in Table 1. Since the crude protein content of APC was higher than that of skim milk, less APC was needed to replace a given amount of milk protein. To balance the rations the fat and glucose contents were increased with increasing AP substitution levels.

Three Holstein male calves, from three to five days of age were allocated to each treatment. The calves were housed in individual pens under a shelter, with sides open. The calves were changed from whole milk to the milk replacers. within three days and were given

Table 1. The ingredients and chemical composition of milk replacers.

Ingredient	0 % AP	25 % AP	50 % AP	75 % AP	100 % AP
Non-fat dry milk	58.00	42.00	29.52	12.00	–
Pro-Xan	–	10.52	18.40	28.40	35.28
Fat premix[1]	24.00	24.00	26.80	29.20	34.60
Glucose[2]	13.72	19.20	21.00	24.12	25.84
Mineral premix	1.24	1.24	1.24	1.24	1.24
Def. rock phosphate	2.40	2.40	2.40	2.40	2.40
Vitamin premix[3]	0.40	0.40	0.40	0.40	0.40
Antibiotic[4]	0.24	0.24	0.24	0.24	0.24
			Chemical composition		
Dry matter (%)	93.4	93.2	94.0	93.8	93.5
Crude protein (%)	23.2	23.7	23.4	23.8	23.5
Ether extract (%)	8.6	11.4	12.1	13.9	15.7
Gross energy (cal/g)	4.522	4.632	4.749	4.947	5.061

[1] FM 7-40-60, Land O Lakes Minneapolis, Minn.

[2] Cerelose, Corn Products Company International,. Englewood Cliffs, NJ.

[3] Turkey Starter Premix, kindly supplied by Hoffmann-LaRoche Inc., Fresno, CA.

[4] Aureomycin 10, contained 22 g chlortetracycline per kg, American Cyanamid Co., Princeton, NJ.

two equal feedings daily. The amount of the liquid milk replacers fed was calculated at 7.5 % of the body weight and the dry matter concentration of the reconstituted liquid was 8 % at the start of the experiment. The liquid rations were gradually increased to 10 % of the body weight and 15 % dry matter concentration over a period of 14 days. Body weights were taken at weekly intervals. The liquid diets were prepared just prior to each feeding. The calves were gradually weaned in the 6 th week and the experiments were terminated when the calves reached eight weeks of age.

In addition to the liquid milk replacers, the calves on growth trials received alfalfa hay and a commercial calf starter. Hay was provided free of choice but the maximum consumption of calf starter was limited to two kg per calf per day. In digestibility trials the calves received only reconstituted milk replacers for the first five weeks of the experiment. At the end of the third week the calves were placed in digestion trial crates for separate collections of feces and urine. After a three day adjustment period, total collections were obtained for six days.

In assessing health of the calves a scoring system was used to classify feces consistency, dehydration, calf demeanor and appetite. The scores ranged from one to four. For feces consistency, rank one was given to normal, formed feces while rank four indicated watery feces.

Similar scoring scales were used for daily ratings of dehydration, demeanor and appetite.

The precipitation speed of APC particles in reconstituted milk replacers was measured in 2.5 cm diameter, 100 ml graduated cylinders as the height of column precipitate. In vitro rennet coagulation was measured in a 100 ml reconstituted sample of milk replacers at 10 % concentration in a water bath at 38°C. A standard cheese making rennet of 0.2 ml was used. Standard statistical techniques and Student–Newman–Keul's multiple range test were used to analyze the data.

Results and discussion

Growth trials: The performance data of the calves in the first five weeks are summarized in Table 2. The among group differences for all the variables in growth trials were not statistically significant. The average daily gains (ADG) for the 0, 25 and 50 % AP groups were within the range of 450–519 g. Then, as the level of substitution was increased, the ADG decreased to 329 and 377 g for the 75 and 100 % substitution groups, respectively. The ADG by calves fed APC at up to 50 % replacement levels were higher than, and at 75 and 100 % levels were similar to, the values reported for comparable experiments run elsewhere, where fish milk, soybean, and dried microbial cell proteins were used (2, 3, 4). Two calves on the 75 % AP level developed a respiratory problem which slowed down their growths.

Tablo 2. Average performances of calves in the first five weeks.

Criterion	0 % AP	25 % AP	50 % AP	75 % AP	100 % AP	Standard error
			Growth trials			
Initial weight (kg)	43.3	44.9	43.5	46.4	45.2	3.04
Fifth week weight (kg)	60.3	60.6	61.3	57.9	58.4	4.27
Average daily gain (g)	519	450	506	329	377	66.2
Daily replacer powder (g)	543	557	546	563	549	11.1
Daily calf starter (g)	386	183	411	85	106	60.9
Daily alfalfa hay (g)	243	220	269	269	143	44.0
			Digestibility trials			
Initial weight (kg)	34.6	35.6	36.8	35.3	36.2	1.88
Fifth week weight (kg)	42.2	43.1	43.4	41.6	40.9	1.91
Average daily gain (g)	220a	216a	187a	180a,b	134b	16.2
Daily replacer powder (g)	563	591	600	577	603	12.7

a,b Means with unlike superscripts within rows are different ($P < 0.05$).

The average values for the consumption of milk replacer powder, calf starter and alfalfa hay were similar. The calves tended to consume less feed dry matter as the levels of AP replacement were increased.

The average values of the health scores for the various diets during the nursing period are given in Table 3. Since the differences between growth and digestion trials were not statistically significant the data for the growth and digestion trials were combined within the substitution levels. The average values were significantly ($P < 0.05$) different for the dehydration and demeanor scores, but the differences among the means were not consistent with the change of the substitution level. Average appetite scores were all close to one (voracious) which indicated that the calves readily accepted APC in milk replacers. The adjustment of the calves to the milk replacers resulted, in some cases, in digestive disorders, reduced growth rate, muscular weakness and respiratory problems in the first two weeks. After the adjustment all of these symptoms disappeared.

Table 3. Visual evaluations of calves in both trials in the first five weeks.

Observation	0 % AP	25 % AP	50 % AP	75 % AP	100 % AP	Standard error
Days scouring/calf	6.83	4.67	7.00	4.00	4.80	1.21
Feces	1.95	1.87	2.03	1.70	1.75	0.12
Dehydration score	1.21^{ab}	1.38^{a}	1.06^{b}	1.18^{ab}	1.26^{ab}	0.06
Demeanor score	1.10^{ab}	1.22^{ab}	1.08^{b}	1.12^{ab}	1.34^{a}	0.06
Appetite score	1.10	1.12	1.06	1.14	1.10	0.07

a,b Means with unlike superscripts within rows are different ($P < 0.05$).

The feeding and management of the calves on both growth and digestibility trials were the same after weaning (6 to 8 weeks) period. Although the average daily gain at the 0 % replacement level appeared greater than the others, neither trial nor substitution level was significantly different. The post weaning growth rates of the calves were comparable to the reports in the literature (3, 4). The calves were generally healthy and none of them scoured during this period.

Digestion trials: Since no other dry feed was given in the first five weeks period, the rate of gains of the calves were small; only approximating those for the growth trial (Table 2). The growth rates of the calves decreased significantly ($P < 0.05$) as the AP substitution level

was increased. However, the multiple range test indicated that the differences among the average values of up to the 75 % substitution level were not significant. The growth rates in this investigation were higher than the average values reported by others on alfalfa protein coagulum (10).

In most veal calf oparations skim milk-based milk replacers are used in the first 3–4 weeks and then the calves are changed to less expensive, partly soy-based preparations. In this experiment the AP milk replacers were started at 3–5 days of age. Since the growth response was not severely affected, even at such an early age, it appears that up to 50 % AP milk replacers could be used for raising replacement and veal calves. The association between substitution level and average daily gain is linear and the average daily gain decreased 1.99 g for every 1 % increase in AP substitution.

The average digestibility and nitrogen retention values are presented in Table 4. The average DM digestibility for the 0 % AP level was 90.6 %. As the substitution level was increased, the average DM digestibility decreased, eventually reaching 72.5 % for the 100 %

Table 4. Digestibilities and Nitrogen retention by calves fed only milk replacers.

Criterion	0 % AP	25 % AP	50 % AP	75 % AP	100 % AP	Standard error
			Digestibilities (%)			
Dry matter	90.6^{a}	86.0^{b}	83.1^{b}	76.6^{c}	72.5^{d}	1.79
Apparent nitrogen	85.4^{a}	78.4^{ab}	77.5^{ab}	67.4^{b}	60.5^{bc}	2.63
Gross energy	91.3^{a}	85.8^{ab}	83.5^{b}	76.0^{c}	71.6^{c}	2.01
Nitrogen free extracts	95.4^{a}	91.4^{b}	88.6^{b}	83.3^{c}	82.0^{c}	1.38
Ether extracts	95.6^{a}	91.8^{ab}	91.8^{ab}	86.4^{b}	87.8^{b}	1.11
			Nitrogen retention			
N intake (g/day)	24.25	24.69	24.63	25.01	24.62	0:12
Fecal N (g/day)	3·53	5·33	5·54	8.15	9.72	1.18
Urine N (g/day)	8.64	8.30	9.99	8.48	8.76	1.10
N retention (g/day)	12.57^{a}	11.06^{ab}	9.09^{ab}	8.38^{ab}	6.14	0.78
Retention of N intake (%)	51.8^{a}	44.8^{ab}	36.9^{ab}	33.5^{ab}	24.9^{b}	3·19
Retention of absorbed N (%)	60.6	57.1	47.9	48.5	41.0	3.11

a,b,c,d Means with unlike superscripts within rows are different ($P < 0.05$).

AP level (Figure 1). The differences among the means were significant ($P < 0.01$). The regression of DM digestibility on level on substitution was linear and the regression coefficient was negative (Table 5). The differences among the means for apparent nitrogen digestibility were again highly significant ($P < 0.01$). However, according to the mul-

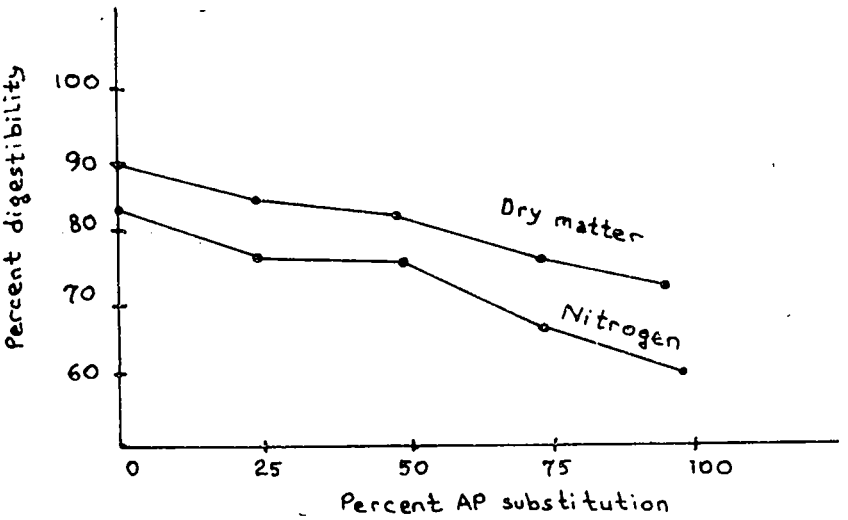

Figure 1. Apparent digestion coefficients of dry matter and nitrogen.

Table 5. Regression coefficients and coefficien of determinations of nutrient digestibilities and N balance on AP substitution level.

Criterion	b ∓ SE	r^2
Dry matter digestibility	−0.39∓0.28	0.93
Nitrogen digestibility	−0.88∓0.69	0.80
Nitrogen balance	−0.17∓0.30	0.58
Gross energy digestibility	−0.55∓0.43	0.87
NFE digestibility	−0.33∓0.21	0.93
EE digestibility	−0.57∓0.40	0.62

tiple range test the specific differences among the levels of up to 50 % AP substitution were not statisticaliy significant. The rate of decline increased after the 50 % level. The regression of nitrogen digestibility on the substitution level was linear and the regression coefficient was negative. The coefficient of determination indicated that 80 % of variation in apparent nitrogen digestibility was accounted for by the variation in AP substitution level. Such decrease in nitrogen digestibility has been the common result in all the previous reports where increasing levels of non-milk proteins were used (4, 8). However, non-significant differences among the 0, 25 and 50 % AP substitution levels were encouraging. The differences among the means for gross energy digestibility were again statistically significant ($P < 0.01$) and the declining trend was similar to that of dry matter digestibility.

The differences among the means for nitrogen retention were significant ($P < 0.05$) only between 0 and 100 % AP substitution levels. Among level differences, on the other hand, were not significant for the retention of absorbed nitrogen. Since the average values up to the 50 % substitution level were not significantly different, it could be interpreted that AP has a good potential as a substitute for milk protein in milk replacers. Some improvements can possibly be obtained with finer grinding and a different processing method which would promote higher solubility of APC.

Physical adn chemical constants: A good substitute for milk protein should possess good suspension characteristics in water. The Pro–Xan used was a heat precipitated protein and it settled in the bottom of the nursing bottle within 20 minutes after the milk replacer was reconstituted.

With the rennet coagulation tests, complete coagulation occurred at 0 and 25 % substitution levels within two minutes. As the level of substitution increased, the complete coagulation time became longer, the clots were less distinct, and some precipitation was formed on the bottom of the beaker. At the 100 % substitution level complete coagulation did not occur. Some flocculent curds formed in the beaker and a precipitate formed on the bottom.

Pro–Xan, with its present precipitation rate, could cause practical feeding difficulties in commercial application. However, the results obtained from this investigation suggest that soluble proteins from alfalfa juice deserves to be tested in calf milk replacers.

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