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Abstract

Alfalfa (*Medicago sativa*) is a high biomass perennial legume that is grown across the U.S. for use in animal feeds. Yield and composition of protein concentrates extracted from fresh alfalfa foliage were measured using five methods of protein precipitation. The acid based methods resulted in the highest yield and lowest fiber content, while heating resulted in a higher protein concentration. Heat treatment resulted in increased concentration of limiting amino acids and lower sugar content compared to the other methods. Using heat treatment total foliage yielded an average of 105 g protein concentrate/kg dry matter. A commercial alfalfa protein concentrate was used to replace fishmeal in diets for yellow perch (*Perca flavescens*) and rainbow trout (*Oncorhynchus mykiss*). Yellow perch on the control diet had a higher weight gain and growth rate but feed conversion ratios, fillet yield and fillet composition was similar on both diets. For rainbow trout, there were no significant differences in growth performance or fillet composition by diet. Thus, alfalfa protein concentrate had reasonable palatability as a fishmeal replacement for yellow perch and rainbow trout.

Introduction

- Plant-based non-GMO sources of protein are needed for replacing fishmeal in aquaculture diets. Most replacements in current use are derived from seeds, and often contain undesirable anti-nutritional components.
- Alfalfa is a high biomass perennial legume widely planted across the United States. It is used to feed livestock due to its high crude protein concentration. Cultivation of alfalfa also has numerous environmental benefits including protecting water quality and providing nectar for pollinators.
- Alfalfa protein concentrate (APC) extracted from “juicing” alfalfa foliage has a balanced amino acid profile, and also contains carotenoids and omega-3-fatty acids, making it an attractive component for aquaculture feeds.

The goals of this study were to:

- Measure yield and composition of APC from total alfalfa forage using different extraction methods.
- Determine growth and composition of yellow perch and rainbow trout fed diets containing APC.

Table 1. Formulation of diets.

Diet	% CP	% CL	% DP	% FM	% APC	% FO	% SBM	DE (kcal/g)
Yellow perch control	40.4	12.0	36.8	15.0	0.0	6.3	15.0	4.08
Yellow perch APC	40.1	12.0	36.7	0.0	18.4	5.3	15.0	4.02
Rainbow trout control	39.0	19.0	34.6	15.0	0.0	8.0	16.0	4.15
Rainbow trout 3% APC	39.2	19.0	34.7	12.9	3.0	8.0	16.0	4.15
Rainbow trout 6% APC	39.4	18.9	34.9	10.9	6.0	8.0	16.0	4.15

CP: crude protein; CL: crude lipids; DP: digestible protein; FM: fishmeal; APC: alfalfa protein concentrate; FO: fish oil; SBM: soybean meal; DE: digestible energy.



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Methods and Materials

Testing Five APC Extraction Methods

- Plant material was homogenized in water with a blender and pulp hand pressed through cheesecloth to extract juice.
- Protein was precipitated in juice by: (1) heating to 80°C for 30 minutes, (2) freezing at -15°C, (3) adding HCl to pH 4.0 (4) adding HCl to pH 4.0 at 4°, or (5) raising the pH of the juice to 10.0 with NaOH then lowering the pH to 4.0 with HCl.
- Protein was concentrated by centrifugation and dried at 43°C.
- Compositional analysis and amino acid analysis were done by Minnesota Valley Testing Laboratory.

Yellow Perch Feeding Trials

- Diets were formulated with 40% crude protein and 12% crude lipids with APC replacing fishmeal in the test diet (**Table 1**).
- Young of the year were housed in eight 600 L aluminum recirculation aquaponic tanks located within a greenhouse with four tanks receiving each feed over 14 weeks.

Rainbow Trout Feeding Trial

- Diets were formulated for 45% crude protein and 19% crude fat with APC replacing 3 or 6% of the fishmeal in test diets (**Table 1**).
- Fingerlings were distributed in eight raceways with two raceways receiving each feed over 12 weeks

Results

- Yield of APC was highest in the three acid coagulation methods with the freezing and heating methods yielding significantly less APC (**Table 2**).
- Heating resulted in the highest crude protein concentration, followed by freezing and acidification methods.
- Because the total APC yield was highest with acidification, this method yielded the highest total amount of CP per kg starting dry matter.
- All coagulation methods resulted in a low fiber content in the APC products.
- For yellow perch weight gain and growth rate was greatest with the control diet although fillet yield and composition was similar on both diets.
- For rainbow trout there were no differences in growth performance of fillet composition by diet.

Table 2. Alfalfa protein concentrate (APC) yields and contents from five coagulation methods.

	Yield (g/kg dry matter)	% Crude Protein	% Fat	% Sugar	% Fiber	% Limiting AA
Acid	137.5 a	45.8 c	9.0 c	2.0 bc	0.6 bc	5.3 b
Acid + Base	141.7 a	45.3 c	10.1 abc	1.3 cd	0.6 c	5.2 b
Cold Acid	134.2 a	45.5 c	9.6 bc	2.1 b	0.7 abc	5.2 b
Freezing	108.8 b	48.9 b	11.1 ab	3.8 a	1.1 ab	5.3 b
Heating	105.0 b	50.9 a	11.4 a	1.1 d	1.2 a	5.8 a

Limiting AA is the sum of percent methionine, lysine, and threonine in the protein concentrate. Significant differences ($P \leq 0.05$; Tukey's HSD) are indicated by different letters within columns.

Figure 1. Wet protein precipitates.



Figure 2. Performance of yellow perch on a control diet with fishmeal (FM) and alfalfa protein concentrate (ANC). Studies were done for 14 and 16 weeks in 2017 and 2018, respectively. **A**, Feed intake rate as a percentage of body weight. **B**, Specific growth rate.

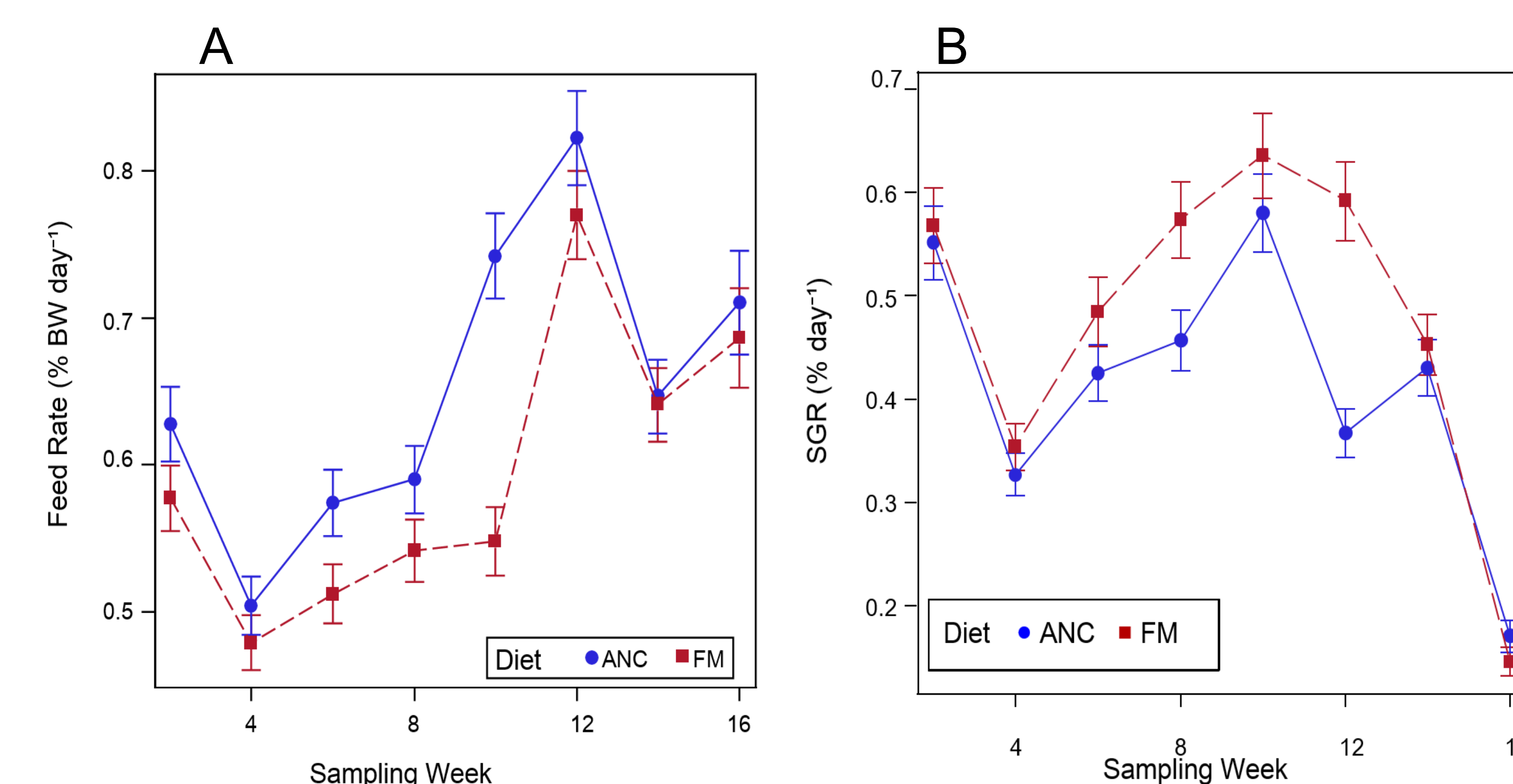


Table 3. Growth performance and fillet composition of yellow perch and rainbow trout on diets replacing fishmeal with alfalfa protein concentrate (APC).

Parameter	Perch Diets		Trout Diets		
	FM	APC	Control	APC3	APC6
Growth Performance Indexes					
Initial Body Weight (g)	23.44a	21.66b	56	51	55
Final Body Weight (g)	40.44a	33.27b	116	113	116
Weight Gain (%)	67.14a	54.67b	108.5	122.3	112.7
Specific Growth Rate (%/day)	0.46a	0.39b	0.79	0.86	0.81
Condition Factor	1.10	1.08	1.21	1.22	1.18
Survival (%)	87	83	ND [†]	ND	ND
Feed Intake (% BW/day)	0.59b	0.65a	0.69*	0.69	0.69
Feed Conversion Ratio	1.67	1.99	0.82	0.91	0.89
Fillet Yield (%)	39.82	39.81	49.48	47.00	48.27
Viscerosomatic Index	7.58b	8.13a	10.88	11.85	11.10
Hepatosomatic Index	2.30	2.40	1.53	1.63	1.57
Fillet Proximate Composition					
Moisture (%)	78.86b	79.26a	75.23	74.16	75.37
Crude Protein (%)	19.63	19.38	17.55	17.60	18.02
Crude Fat (%)	0.16	0.14	4.94	5.89	4.96
Ash (%)	1.28	1.27	1.43	1.53	1.58
Gross Energy (kcal/g)	1.04	1.03	1.42	1.55	1.46

Within a row and species, treatment means with differing letters are significantly different ($P \leq 0.05$).

[†] Not determined. Survival was not recorded by raceway and could not be determined by diet. Overall survival was >98%.

* Feed intake for rainbow trout was averaged across all raceways.

Discussion

- Heating the foliage extract produced the highest concentration of protein and limiting amino acids in the product.
- Slightly different product composition was obtained by different precipitation methods.
- Extraction of APC from reduced-lignin varieties did not result in higher protein extraction compared to conventional varieties (data not shown).
- Yields of APC were similar from total forage and leaves separated from total forage on a dry matter basis (data not shown).
- APC was palatable by yellow perch and rainbow trout although weight gains were highest in control diets with fishmeal for yellow perch.

Conclusions

- APC does not need to be produced from lower yielding immature foliage but can be produced from foliage harvested at later maturities to maximize yields.
- Protein content, amino acid composition and concentration of fatty acids, makes APC a viable component for aquaculture feeds, which will increase the value of harvested alfalfa.
- Feeding studies indicate that APC is palatable by yellow perch and rainbow trout although more research is needed in diet formulation to improve growth performance.