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DIGESTIBILITY OF PEA PROTEIN CONCENTRATE AND ENZYME-TREATED PEA FLOUR IN MILK REPLACERS FOR CALVES

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In an experiment involving enzymatically hydrolyzed pea flour (HPF) and pea protein concentrate (PPC) to provide half the dietary protein in milk replacers, the pea protein was found to be about 25% digestible by calves under 2 wk of age and 65 to 70% digestible by calves 3 wk of age. The younger calves digested 42 to 53% of the pea starch; older calves digested 63 to 84%. The digestibility of dry matter, protein, energy and ether extract increased with calf age ($P < 0.01$). In the second trial, involving PPC at 7.7, 15.4 and 23.1% of the dry matter in milk replacers, and providing 19, 33 and 46% of the dietary protein, the digestibility coefficients for dry matter, disregarding calf age, declined from 90 to 86% as PPC increased from 15.4 to 23.1%, but the low and intermediate PPC diets were equally well digested. The reduction was due mainly to the corresponding reduction in protein digestibility. Digestibility coefficients for dry matter, protein, energy, ether extract and nitrogen-free extract for milk replacer diets containing 23.1% PPC exceeded 81% with calves over 14 days old. The third experiment involved feeding the control milk replacer until the calves were 2 wk old, then commencing the feeding of PPC and HPF replacers, using formulas similar to those of the first experiment. The digestibility coefficients were similar to those of the first experiment, indicating that the observed improvement in older calves was primarily a function of calf age and not markedly influenced by the formula of the diet. These studies indicate that PPC and HPF are not satisfactory ingredients in milk replacers to be fed to calves under 2 wk of age, but, though less digestible than milk, are effectively digested thereafter.

Dans une expérience sur de la farine de pois hydrolysée par enzyme (FPH) et du concentré de protéine de pois (CPP) fournissant la moitié des protéines de la ration dans des aliments d'allaitement, la protéine de pois avait une digestibilité d'environ 25% pour les veaux de moins de deux semaines, et de 65 à 70% pour les veaux de trois semaines. Les très jeunes veaux ont digéré de 42 à 53% de l'amidon de pois, tandis que les plus vieux en ont digéré de 63 à 84%. La digestibilité de la matière sèche, de la protéine, de l'énergie et de l'extrait à l'éther ont augmenté avec l'âge des veaux ($P < 0.01$). Dans la deuxième expérience sur du CPP à raison de 7.7, 15.4 et 23.1% de la matière sèche des aliments d'allaitement de fournissant 19, 33 et 46% de la protéine de la ration, les coefficients de digestibilité de la matière sèche, quel que soit l'âge des veaux, ont diminué de 90 à 86% alors que la teneur en CPP passait de 15.4 à 23.1%, mais les rations à teneur faible et moyenne en CPP ont été également bien digérées. La baisse était due principalement à une baisse correspondante de la digestibilité des protéines. Les coefficients de digestibilité de la matière sèche, de la protéine, de l'extrait à l'éther et de l'extrait non azoté des aliments d'allaitement contenant 23.1% de CPP ont dépassé 81% pour les veaux de plus de 14 jours. La troisième expérience comportait un essai d'alimentation de veaux avec un aliment d'allaitement.

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ment témoin jusqu'à ce qu'ils atteignent l'âge de deux semaines, puis avec des aliments à base de CPP et de FPH de composition semblable à celle de la première expérience. Les coefficients de digestibilité étaient semblables à ceux de la première expérience, ce qui indique que l'amélioration observée chez les veaux plus vieux était principalement fonction de l'âge des veaux et que la composition de la ration n'a pas eu d'effets marqués. Ces expériences indiquent que le CPP et la FPH ne sont pas des composants satisfaisants pour les aliments d'allaitement pour veaux de moins de deux semaines, et que même s'ils sont moins digestibles que le lait, ils ont bien été digérés après cet âge.

A protein concentrate derived from peas (*Pisum sativum*), when supplemented with methionine, was found to equal egg, fish protein concentrate or methionine-supplemented casein in biological value (Bell and Youngs 1970). It was also found to possess physical properties favorable to its use in liquid preparations involving emulsification of fat. (Sumner, A. K., Leinan, L. M. and Youngs, C. G. Food products with high protein content and process for producing them. Can. Pat. Application #143,692. 2 June 1972). Pea protein concentrate (PPC) was therefore indicated as a potential protein source in calf milk replacers.

The incorporation of PPC into milk replacer formulations as a partial replacement for dried skim milk leads to the need to supply a suitable carbohydrate source to replace the lactose. Newborn calves can utilize glucose readily (Dollar and Porter 1957; Radostits and Bell 1970); consequently, a possible means of providing useful carbohydrate would be to enzymatically hydrolyze the starch in pea flour, without separating the flour into PPC and a starch fraction as done by Bell and Youngs (1970).

The experiments described herein were designed to determine the digestibility of PPC and a hydrolyzed pea flour product with calves up to 3 wk of age.

MATERIALS AND METHODS

This study was carried out in three experiments. The initial one consisted of a trial to evaluate field peas as a potential protein and energy source in milk replacers for young calves. Eight calves, four male and four female, were allotted in a simple block design to each of three diet treatments: a control milk-based diet, a diet containing 20% PPC plus cerelose, and a diet containing pea flour hydrolyzed with a glucoamylase (HPF) (Table 1). The milk replacers containing pea products involved replacing half the dietary protein with pea protein.

PPC was prepared by the method of Bell and Youngs (1970). HPF was prepared from peas which had been split in a Burr mill and dehulled with a fanning mill. The dehulled peas were ground in a Burr mill to flour-like fineness and then added to water in a 1:4 ratio by weight. The resultant slurry was passed through a Bauer plate refiner three times and then poured into a steam-jacketed reaction kettle where a starch-thinning enzyme, HT 1000 (Marschall Division, Miles Laboratories Ltd., 77 Belfield Road, Rexdale, Ont.) was added at the level of 0.25% of the weight of the original pea flour. The temperature was held at 95 C for 1 h, then the reaction mixture was cooled to 55 C and a glucoamylase, Diazyme (Miles Laboratories Ltd.), was added. This enzyme was used at 0.5% of the weight of original pea flour. The temperature was maintained at 55 C for 4 h, after which the material was dried on a steam-heated (2.81 kg/cm²), double-drum drier and then in an air-draft oven at 50 C. The dried material was ground in a Wiley mill to a medium-fine flour. The yield from this procedure was 85% of the weight of the original material due to handling losses. Approximately 80% of the starch was hydrolyzed to glucose.

The second trial was conducted to determine the effect of the level of PPC in milk replacer on diet digestibility. Three levels of PPC (7.7, 15.4 and 23.1% of the dry matter (DM); Table 2) were fed to Holstein-Friesian calves in three successive 7-day periods in a latin square design. The experiment was replicated three times employing a total of nine calves.

The third trial was designed to ascertain whether the increase in digestibility of the diets with age, noted in trials 1 and 2, was due to specific adaptation of the calves to the diet or whether basic changes in the digestive physiology of the calves occurred at 10 to 12 days of age, regardless of diet. To test these two alternatives, eight Holstein-Friesian calves were fed the milk-based control diets used in trial 1 until they reached 10 days of age, then were switched to either the PPC plus cerelose or the HPF diets used in trial 1.

All calves were weaned at 3 days of age and housed in individual elevated stalls. The calves

Table 1. Composition of milk replacers used in trials 1 and 3

Ingredient or factor	Diet		
	Control	Pea protein conc. + cerelese	Hydrolyzed pea flour
Premix† (kg)	25.0	25.0	25.0
Skim milk, spray-dried (kg)	37.0	9.0	10.0
Whey, sweet, dried (kg)	37.0	15.0	14.0
PPC (kg)	—	20.0	—
HPF (kg)	—	—	48.0
Cerelese (kg)	—	28.3	—
Methionine (g)	110	350	240
Aureo‡SP250 (g)	250	250	250
Choline (g)	—	134	115
Vitamin A (IU)	243,000	358,000	358,000
Vitamin D (IU)	44,000	88,000	88,000
Vitamin E (IU)	242,000	242,000	242,000
Vitamin B ₁₂ (mg)	2.9	2.9	2.9
ZnO (g)	2.2	4.4	4.4
FeSO ₄ ·2H ₂ O (g)	1.1	1.1	—
FeSO ₄ ·7H ₂ O (g)	—	—	32.6
CuSO ₄ ·5H ₂ O (g)	3.3	0.44	0.44
CaHPO ₄ ·2H ₂ O (kg)	—	1.56	1.34
CaCO ₃ (g)	—	—	540
MnO (g)	—	3.85	3.30
Cobalt-iodized NaCl (g)	—	400	400
Protein N×6.25, (%)	25.3	24.5	26.8
Protein from peas (% of total)	—	50.2	52.0
Nitrogen-free extract (%)	52.6	55.6	54.0

†20% brewers' dried yeast, 40% lard, 33% dried buttermilk and 7% dried whey; homogenized and spray-dried.

‡A mixture of chlortetracycline, sulphamethazine and penicillin, courtesy of Cyanamid of Canada Ltd.

Table 2. Composition of milk replacers used in trial 2

Ingredient or factor	Level of pea protein conc.		
	7.7%	15.4%	23.1%
Premix† (kg)	25.0	25.0	25.0
Whey, sweet, dried (kg)	31.0	23.2	23.2
Skim milk, spray dried (kg)	25.0	25.0	17.3
Brewers' dried yeast (kg)	5.0	5.0	5.0
Pea protein conc. (kg)	7.7	15.4	23.1
Lactose (kg)	5.5	5.5	5.5
Methionine (g)	100	100	100
Aureo SP250 (g)	250	250	250
Vitamin A (IU)	236,000	236,000	236,000
Vitamin D (IU)	44,000	44,000	44,000
Vitamin E (IU)	242,000	242,000	242,000
Vitamin B ₁₂ (mg)	1.2	1.2	1.2
Protein, N×6.25, (%)	26.7	30.6	32.3
Protein from peas (% of total)	19	33	46
Nitrogen-free extract (%)	54.1	50.8	49.1

†40% lard, 60% skim milk, by weight; homogenized and spray-dried.

were fed amounts of the test milk replacer (initially about 680 g/day, diluted 1:8 in warm water) adjusted to their individual appetites, divided into two or three feedings daily. Chromic oxide was used for the determination of the digestibility coefficients and was added to the milk replacer at the time of feeding at a level of 0.5% of the air-dry weight of the replacer. Fecal samples were obtained directly from the rectum, mixed with a preservative (2 g HgCl₂ and 10 g boric acid added to 1,000 ml distilled water) and were stored frozen until analyzed.

Kjeldahl, dry matter (DM) and ether extract (EE) determinations were carried out according to standard procedures of the Association of Official Agricultural Chemists (1960). Gross energy was determined with the Parr oxygen bomb calorimeter. The method of Bolin and Lockhart (1960) was used in the analysis for chromic oxide. Amino acids were measured with the Technicon amino acid analyzer in order to study amino acid digestibility.

Digestibility coefficients were transformed to their respective arcsin $\sqrt{\%}$, and analysis of variance was carried out on the transformed values (Steel and Torrie 1960). Duncan's multiple range test was applied to locate significant differences (Steel and Torrie 1960).

RESULTS AND DISCUSSION

PPC and HPF diets

The calves fed the control diet digested the dry matter (DM) effectively in all test periods (Table 3). Calves fed either the PPC or HPF diets displayed lower DM digestibility ($P < 0.05$) during the 1st 2 wk and the coefficients remained below the control diet until the end of the trial. Similar responses prevailed in regard to crude protein (CP), energy, ether extract (EE) and nitrogen-free extract (NFE) although varying somewhat in magnitude.

The protein in PPC and HPF diets was only about 50% digested by calves up to 2 wk of age and since these diets contained some milk proteins, it is obvious that the pea protein itself was less than 50% digestible. Since about half the protein in these diets was from milk sources, and if it is assumed that the milk protein in the pea protein diets was as well digested as in the control diet, the pea protein can be estimated to be not more than 25% digestible. Similar calculations from the data obtained from calves over 2 wk old showed pea protein to be

Table 3. Coefficients of apparent digestibility for dry matter, crude protein, energy, ether extract and nitrogen-free extract of control, pea protein concentrate (PPC)+cerelose and hydrolized pea flour (HPF) diets (trial 1)

Diet	Calf age in days						Diet means (%)	
	7-9 (%)	10-12 (%)	13-15 (%)	16-18 (%)	19-21 (%)	22-24 (%)		
Dry matter	Control	92	89	87	90	90	93	90 a
	PPC + cerelose	76 b	73 b	86 a	86 a	87 a	87 a	83 b
	HPF	69 b	70 b	81 a	82 a	83 a	82 a	78 c
Crude protein	Control	84	83	83	86	84	88	85 a
	PPC+cerelose	50 b	49 b	74 a	77 a	79 a	79 a	68 b
	HPF	51 b	54 b	72 a	72 a	75 a	73 a	66 b
Energy	Control	91	90	89	90	90	92	90 a
	PPC+cerelose	72 b	65 b	85 a	85 a	86 a	85 a	80 b
	HPF	66 b	69 b	81 a	81 a	83 a	82 a	77 b
Ether extract	Control	91	91	94	96	96	95	94 a
	PPC+cerelose	65 c	48 b	83 a	82 a	82 a	84 a	74 b
	HPF	46 b	56 b	82 a	87 a	86 a	89 a	74 b
Nitrogen-free extract	Control	97 a	95 ab	94 b	95 ab	95 ab	96 ab	95 a
	PPC+cerelose	91 b	93 ab	94 ab	94 ab	94 a	94 ab	94 b
	HPF	84 b	84 b	87 ab	88 ab	89 a	88 ab	87 c

a, b Means without letters, or with similar ones, are not significantly different, $P < 0.05$.

Effect of calf age comparisons in rows; diet comparisons in last column. Significance of interaction means is not shown; that is, diet comparisons within age groups.

about 65 to 70% digestible. Similar age-related increases in digestive ability in young calves were reported by Noller et al. (1956), also working with vegetable proteins.

The digestibility of the carbohydrates, as indicated by DM and by NFE, revealed those in HPF to be less digestible than in the PPC diets. Cerelose (largely glucose) is well utilized by the young calf (Huber et al. 1961b; Okamoto et al. 1959) and there was only about 5% starch in the PPC diets introduced as part of the PPC. This is in accord with the 91% digestibility of NFE in the PPC diet compared with 95% for the control, with 7 to 9-day-old calves. The HPF diet was calculated to contain about 12% carbohydrate more complex than glucose, and the 84% digestibility coefficient again confirms the well established inability of the newborn calf to digest starch (Radostits and Bell 1970), but also indicates that the pea starch that had been hydrolyzed to glucose during the manufacturing of the HPC was readily digested.

If it is assumed that glucose and milk sugars were as well digested in the pea diets as in the control diet, it can be estimated that 7- to 9-day-old calves digested 42 to 53% of the complex pea carbohydrates in HPF and PPC, respectively. The corresponding coefficients for 13- to 24-day-old calves were 63 and 84%. These observations agree well with the findings of Huber et al. (1968) with calves between 10 and 24 days of age, and with the data of Raven and Robinson (1958).

Morrill et al. (1970) also found that when Diazyme L 30 (Miles Laboratories Inc.) was added to milk replacers containing soybean starch that was fed immediately, calf responses were similar to responses obtained when glucose was fed.

The digestibility of the DM in the milk replacers, (Table 2) disregarding calf age, was depressed ($P < 0.05$) from 90 to 86% (Table 4) by increasing the PPC content from 7.7 to 23.1%. However, the depressing effects were significant at only about the

Table 4. Coefficients of apparent digestibility for dry matter, crude protein, energy, ether extract and nitrogen-free extract for diets containing three levels of PPC (trial 2)

	PPC in diet (%)	Calf age in days			PPC level means (%)
		7-10 (%)	14-17 (%)	21-24 (%)	
Dry matter	7.7	84	93	92	90 <i>a</i>
	15.4	84	92	93	90 <i>a</i>
	23.1	78	89	91	86 <i>b</i>
Mean		82 <i>b</i>	91 <i>a</i>	92 <i>a</i>	—
Crude protein	7.7	67	87	87	80 <i>ab</i>
	15.4	74	87	88	83 <i>a</i>
	23.1	63	81	85	76 <i>b</i>
Mean		68 <i>b</i>	85 <i>a</i>	87 <i>a</i>	—
Energy	7.7	81	93	92	89 <i>a</i>
	15.4	82	93	92	89 <i>a</i>
	23.1	74	89	90	84 <i>b</i>
Mean		79 <i>b</i>	92 <i>a</i>	92 <i>a</i>	—
Ether extract	7.7	74	96	95	88
	15.4	69	95	94	86
	23.1	59	92	94	82
Mean		68 <i>b</i>	95 <i>a</i>	94 <i>a</i>	—
Nitrogen-free extract	7.7	94	97	95	95 <i>ab</i>
	15.4	96	97	96	96 <i>a</i>
	23.1	92	95	96	94 <i>b</i>
Mean		95	96	96	—

a, b Means without letters, or with similar ones, are not significantly different, $P < 0.05$.

15.4% level of PPC. Similar trends were observed for all the major components of DM, although the depression in ether extract (EE) digestibility was not significant ($P = 0.05$).

Calves showed improved digestive ability with advancing age in terms of DM, crude protein (CP), energy and EE, but not NFE. The differences observed in calves with the three age groups, in response to different levels of PPC, were not significant ($P = 0.05$). However, it is recalled that EE and CP were 91 and 84% digestible, respectively, in the control formula (Table 3) for calves of a similar age, compared to 68% for both ingredients in this experiment. In view of these observations, the results of this experiment appear to confirm the low digestibility of PPC by young calves (7–10 days old) and they indicate that the depression in DM digestibility was associated primarily with the reduction in protein digestibility per se. The residual starch in the PPC does not appear to have been particularly deleterious.

Digestibility of Milk Replacers Containing Pea Products Following the Feeding of Control Milk Replacer for the First Two Weeks

The digestibility coefficients of experiment 3 (Table 5) corresponded closely with those obtained with calves of the same age in the above trial (Table 3) and therefore indicate

that failure to "challenge" the digestive system with pea ingredients during the 1st 2 wk had no subsequent adverse effect on ability to digest pea components when introduced at the 2nd wk. Thus, the gradually improving ability to digest the non-milk constituents is more probably age-dependent rather than diet-induced or influenced. This agrees with the findings of Huber et al. (1961a).

Apparent Digestibility of Amino Acids

Amino acid digestion coefficients, calculated from data in experiments 1 and 2 increased with calf age ($P < 0.01$) as did protein, although there were several exceptions as indicated by diet \times age interactions, mostly associated with the relatively high digestion coefficients for milk protein.

There were no significant ($P = 0.05$) differences between diets in trial 2 (Table 6; 7.7, 15.4 and 23.1% PPC), due in part perhaps to high experimental variability as indicated by coefficients of variation for the various amino acids ranging between 9 and 33%.

The amino acid digestibilities in the HPF diets were generally low (Table 6) and in line with the protein digestibility. However, lysine and arginine appeared to be somewhat more digestible.

CONCLUSIONS

The reduction found in DM and energy digestibility of PPC and HPF diets is attribut-

Table 5. Effect of age on coefficients of apparent digestibility for dry matter, crude protein, energy, ether extract and nitrogen-free extract in calves fed PPC + cerelese or HPF diets after receiving the control diet until 2 wk of age (trial 3)

Diet	Calf age in days			Mean	
	14–16	17–19	20–22		
Dry matter	PPC+cerelese	85	88	89	87 <i>a</i>
	HPF	80	80	80	80 <i>b</i>
Crude protein	PPC+cerelese	75	79	81	79 <i>a</i>
	HPF	73	72	70	72 <i>b</i>
Energy	PPC+cerelese	83	87	88	86 <i>a</i>
	HPF	81	80	80	80 <i>b</i>
Ether extract	PPC+cerelese	80	80	88	83
	HPF	80	79	78	79
Nitrogen-free extract	PPC+cerelese	93	95	95	94 <i>a</i>
	HPF	86	87	88	87 <i>b</i>

Age effects were not significant, $P > 0.05$.

Table 6. Apparent digestibility of amino acids by the young calf

	Diet					
	Milk control (%)	7.7% PPC (%)	15.4% PPC (%)	20% PPC+ Cerelese (%)	23.1% PPC (%)	HPF (%)
Aspartic	83	84	84	70	76	64
Threonine	85	81	81	60	71	53
Serine	86	85	85	68	76	64
Glutamic acid	90	89	89	75	83	79
Proline	94	79	90	77	84	72
Glycine	78	70	74	64	66	51
Alanine	82	66	71	65	59	48
Valine	85	82	80	64	74	62
Methionine	91	71	74	88	59	67
Isoleucine	87	82	83	68	73	57
Leucine	89	83	85	70	76	66
Tyrosine	88	82	84	67	74	63
Phenylalanine	84	81	82	66	70	46
Histidine	90	90	87	85	80	73
Lysine	93	85	74	78	81	83
Arginine	83	83	83	83	85	82
Crude protein	85	80	83	68	76	66

able mainly to the poor digestibility of the protein fraction and secondarily to the small amounts of starch or partially hydrolyzed starch, respectively, in the two pea diets.

Pea protein is not effectively utilized because of low digestibility by calves under 2 wk of age, and is probably not over 70% digestible by calves 3 wk old. It was shown that pea starch is equally poorly digested during the 1st 2 wk, but rose substantially by the 3rd wk of age. Enzyme hydrolysis of the pea starch prior to feeding converted about 80% of the starch to glucose, which was easily digested.

These studies indicate that PPC and HPF, as prepared for these formulas, are not well enough digested by calves under 2 wk of age to be recommended as ingredients in milk replacers. However, the increased digestive powers of the calf at 2 or 3 wk of age result in 70 and 80% digestibility of protein and starch, respectively; thus, such pea products could be included in formulas for older calves.

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