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ENERGY, PROTEIN, AND AMINO ACID LEVELS

IN THE RATIONS OF WEANLING PIGS

by

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ABSTRACT

Experiments were designed to study fat, lysine, and methionine additions to rations containing three levels of crude protein for weanling pigs from 3 to 9 weeks of age. The experiments were 3 x 5 x 2 x 2 factorials in which the factors were fat level, protein treatment, breed group, and sex. The levels of supplemental fat were 0, 4, and 8 per cent. The five protein treatments were based on rations containing 12, 16, or 20% protein and 12 or 16% protein supplemented with lysine and methionine so that the level of these amino acids was calculated to be the same as in the 20%-protein ration. The two breed groups were Lacombe x Yorkshire and Hampshire x Lacombe-Yorkshire.

Increasing protein from 12 to 16% resulted in doubling the rate of gain (from 0.11 to 0.22 kg/day) while increasing protein from 16 to 20% resulted in a smaller but significant increase (from 0.22 to 0.28 kg/day). Increasing the lysine and methionine in the 12 and 16% rations resulted in a 27 and 36% increase in rate of gain, respectively. Average daily feed intake was increased when the protein level was increased from 12 to 16%, but the increase in feed consumption was not significant when protein was raised from 16 to 20 per cent. Supplemental lysine and methionine added to the 12 and 16%-protein rations resulted in a 13% increase in daily feed intake. Efficiency of feed utilization was improved ($P < 0.05$) as protein was increased from 12 to 16% (2.97 and 2.14 kg feed/kg gain, respectively) and supplementing these rations with lysine and methionine resulted in a further improvement of 15 and 17% in the efficiency of feed utilization.

Apparent digestible energy for 6-week-old pigs was not significantly influenced by any of the treatment factors. Apparent nitrogen digestibility and nitrogen retention were significantly ($P < 0.05$) increased by supple-

menting the 12 and 16%-protein rations with lysine and methionine. There was a nonsignificant increase in nitrogen retention as the protein level of the ration increased.

A digestion experiment using Cr_2O_3 as an indicator in the feed was carried out when the pigs were 9 weeks of age. As with 6-week-old pigs, apparent digestible energy was not significantly affected by any of the treatment factors. Apparent digestible nitrogen was significantly higher in the 20%-protein ration (70.9%) than it was in the 12%-protein ration (60.6%). Digestibilities of both energy and protein were found to be lower for 9-week-old pigs than for 6-week-old pigs.

The addition of fat to the rations for a 6-week-period was found to reduce serum protein levels in female pigs. Serum protein levels for females were 5.77, 4.79, and 4.74 g/100 ml for the pigs fed 0, 4, and 8%-fat rations, respectively. For males the comparable values were 5.02, 5.18, and 5.00 g/100 milliliters.

These results indicate that, for early weaned pigs, protein may have more effect on feed intake and productive efficiency than does the energy in the ration. The results also show that protein savings can be made by increasing the levels of limiting amino acids in the rations.

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INTRODUCTION

It has been observed that, in general, within physiological limits, animals eat to meet their energy requirements. If it is true that the caloric density or available energy content of a ration affects the amount of ration consumed and thereby the amount of nutrients ingested, then it is reasonable that nutrients should be supplied in a ration in relation to its caloric density.

Feeding standards are available, for various species, which list what is thought to be the minimum requirement for specific nutrients. It has been shown, however, that the requirement for protein can sometimes be reduced if the correct level of essential amino acids and the correct balance of essential amino acids to each other and to nonessential amino acids are available. Lysine and methionine are frequently the most limiting amino acids in the rations of young growing swine. The manufacture of these amino acids at reasonable prices has stimulated research in the area of amino acid supplementation of low-protein rations.

The use of animal fat as a concentrated energy source in higher-energy rations for young growing swine is a common practice. Increases in rate of gain, efficiency of feed utilization, and nitrogen digestibility have been reported when fat was added to rations for growing swine.

The studies reported in this thesis were conducted during 1965 and 1966 to determine the effects of varying fat levels, and lysine and methionine levels in rations containing three levels of crude protein when these rations were fed to weanling pigs from 3 to 9 weeks of age. The criteria of measurement were rate of gain, feed consumption, efficiency of feed utilization, energy and nitrogen digestibility, nitrogen retention, and serum protein level.

LITERATURE REVIEW

In the literature of the last several years, there are numerous reports of energy-nutrient relationships having an effect on the productive performance of several species. Protein-energy interactions have probably received the most study, although amino acid-energy relationships have also been investigated. The effect of differing protein levels and amino acid levels in the rations of swine and other species has been investigated in an attempt to elucidate the requirements that these animals have for these nutrients. A school of thought has developed which feels that nutrient requirements should be expressed in relation to the energy content of the feed in which the nutrient is present.

Protein Levels

A number of workers have shown that the level of protein in the ration of baby pigs and early weaned pigs affects the rate of gain and efficiency of feed utilization. These workers have also shown that the protein requirement changes with the age and weight of the animals. For 2-day-old pigs, protein requirements in excess of 30% have been reported by Sewell et al. (1953) and Reber, Whitehair, and MacVicar (1953); although Becker, Ullrey, and Terrill (1954) suggest that 22% protein of high quality gave good results. For pigs weaned at 1 to 3 weeks of age, an optimum protein level of between 20 and 25% has been reported by Lloyd and Crampton (1958, 1961); Rutledge, Hanson, and Meade (1961); and Blair (1961). Although Peo et al. (1957) found 30% protein produced the fastest gains in pigs weaned at 1 week and fed for 2 weeks, 20% protein was found to be optimum for pigs fed from 1 to 5 weeks of age.

Levels of 18 to 20% protein have been reported as satisfactory for pigs 6 to 9 weeks old (Reber et al., 1953; Lloyd and Crampton, 1958, 1961; Rutledge et al., 1961). Becker et al. (1954) reported that 12% of high-quality protein gave satisfactory growth for pigs from 5 to 9 weeks of age.

Smith and Lucas (1957) reported that 18% protein was adequate for pigs weighing 25 to 50 lb., as did also Blair (1961) and Jones et al. (1962). Aunan, Hanson, and Meade (1961) reported no difference in performance of pigs weaned at 8 weeks when they were fed 14, 16, or 18%-protein rations to 125 lb. liveweight.

Some workers have found that the quality of the protein, i.e. the balance and level of essential amino acids, may influence the growth and feed efficiencies as much as the actual protein level (Becker et al., 1954; Kropf et al., 1959; Blair, 1961; Clawson, Barrick, and Smart, 1963).

Fat Additions to Pig Rations

Most research concerning fat levels in pig rations has been in conjunction with varying protein levels in the rations.

Baby pig rations

Catron et al. (1953) found that some fat was necessary in a simulated milk diet for baby pigs which had received no colostrum. Fat additions have been reported to result in increased gains (Catron et al., 1953; Sewell, Thomas, and Price, 1961) and improved efficiency of feed utilization (Kropf, Pearson, and Wallace, 1954; Crampton and Ness, 1954; Sewell et al., 1961; Lowrey et al., 1963). Opposing reports have been published by Peo et al. (1957), Asplund, Grummer, and Phillips (1960), Manners and McCrea (1963), and Eusebio et al. (1962, 1965) who reported no significant increases in gains or improvement in feed:gain ratio due to fat additions.

Lowrey et al. (1963) have reported that the effect of fat can be influenced by protein quality. Fat additions to a casein diet resulted in increased gains while similar additions to a gluten diet resulted in depressed growth.

Growing pig rations

Kuryvial (1961) reviewed the literature up to that date and stated that conflicting experimental evidence existed concerning the value of adding fat to growing and finishing swine rations. He also reported that 15 or 30% supplemental fat added to the ration used in his studies resulted in increased gains and improved efficiency of feed utilization (Kuryvial, Bowland, and Berg, 1962). Similar results have been reported by Clawson et al. (1962); Bayley and Lewis (1963); Greeley, Meade, and Hanson (1964); Seerley, Poley, and Wahlstrom (1964); and Lodge, Lamming, and Lewis (1965).

Protein-Energy Relationships in Pig Rations

Baby pig rations

Manners and McCrea (1963) have summarized the results of some studies on the protein requirements of baby pigs. They reported that the findings of Becker et al. (1954), which showed a requirement of 24% protein, agrees well with their observations of a requirement of 25% protein when low-fat diets were fed (Manners and McCrea, 1962). They have also found that the optimum protein level for growth was 31% when 21% dietary fat was supplied, which agrees well with the results of Sewell et al. (1953) who listed 32% protein as optimum when 25% lard was added, and with Reber et al. (1953) who listed 41% protein for diets containing 35 to 49% lard. Manners and McCrea (1963) state that "all these results are consistent with the hypothesis that the protein requirement of baby pigs varies according to the caloric density of the diet".

Hare, Reynolds, and Luther (1956) found that increasing productive energy decreased growth when added to low-protein rations and increased growth when added to high-protein rations.

Bowland (1964a) reported a protein x fat interaction in which high fat levels increased gain more on low-protein rations than did low fat levels. He also reported that the calorie:protein ratio may affect energy consumption, particularly when high-fat diets are fed to 3 to 10-week-old pigs.

Lowrey et al. (1962), studying protein deficiency symptoms in early weaned pigs, found that the pigs consumed similar amounts of a 5%-protein diet (casein) whether 3 or 23% fat was added, and that both groups gained equally well up to 11 weeks.

Salmon-Legagneur, Jouandet, and Aumaître (1964) observed that protein intakes and gains decreased as the energy:protein ratio increased. Sewell et al. (1961) reported that equal gains were achieved with rations containing calorie:protein ratios of 70:1 and 50:1 (ratio of productive energy to total protein), although the narrower ratio produced more efficient gains. They found no protein x fat interaction which agrees with the findings of Peo et al. (1957).

Growing pig rations

Clawson et al. (1962) reported that for 8 to 12-week-old pigs a narrow energy:protein ratio (26:1 vs. 31:1 or 38:1 calculated as gross calories per gram of protein in the ration) increased growth and feed efficiency. This effect was not apparent at market weight. They also reported that the narrow protein ratio depressed protein efficiency. A similar result was reported by Bowland (1964b) in the starting-growing phase. Clawson et al. (1962) concluded that added fat aggravated a

protein deficiency by causing a reduction in total food intake. Lowrey, Pond, and Maner (1958); Barnes et al. (1959); Clawson (1960); Noland and Scott (1960); and Pond, Kwong, and Loosli (1960) have all observed a growth depression when fat was added to low-protein diets but not when fat was added to high-protein diets.

Costain and Morgan (1961) have observed another type of protein-energy relationship and reported that gains of growing pigs were significantly higher with high-protein, low-energy rations than they were with high-protein, high-energy rations. Robinson, Morgan, and Lewis (1964) also observed better gains with high protein when fed with lower energy levels (i.e. with 2860 kcal digestible energy (DE) per kg rather than with 3300 or 3080 kcal DE per kilogram). They suggested that for growing pigs the optimum appeared to be 17 to 18% protein with between 2860 and 3080 kcal DE per kg of feed.

Kennington, Perry, and Beeson (1958); Wagner et al. (1963); and Henry and Rrat (1964) all reported no significant energy-protein interaction when feeding growing pigs.

Amino Acid Supplementation

For a general outline of the principles of amino acid supplementation of rations the reader is referred to Rosenberg (1959). Clawson et al. (1962) stated that proper adjustment of amino acid ratios makes it possible to obtain rapid growth from rations containing a much lower level of protein than was recommended at that time. Most of the work in this area has been done on growing pigs and with all vegetable diets.

Bowland and Asplund (1960) reported that lysine additions to a basal wheat ration containing 10% protein, when the ration was fed to baby pigs, increased gains, but that methionine and tryptophan additions to the

lysine-supplemented diets did not further improve performance.

Long et al. (1962) and Meade et al (1965) were both unable to show a growth response when lysine was added to a 16%-corn-soybean meal starting diet. Long et al. (1962) found added lysine depressed growth of pigs from 3 to 5 weeks of age, but they added lysine at what would be considered excessive levels. They did observe increased gains, nitrogen digestibility, and nitrogen retention with added methionine. Meade et al. (1965) were unable to show any effect due to methionine alone.

Lysine requirements for weanling-growing pigs of between 0.50 and 0.75% of the diet have been reported by Germann, Mertz, and Beeson (1958) and Meade, Dukelow, and Grant (1966); although higher levels have been found to be necessary by Evans (1958); Chance et al. (1958); Costain and Morgan (1961); Crehan, Lodge, and Lewis (1964); and Mitchell et al. (1965). Homb (1963) stated that most of the work with weanling pigs points to a requirement of 0.6 to 0.7% of the ration. The 1964 revision of "Nutrient Requirements for Swine" (National Academy of Sciences — National Research Council (US)) listed 0.75% L-lysine with 18% protein for growing pigs and about 1.07% L-lysine with 22% protein for pigs weighing 10 to 25 lb. (NRC, 1964).

Berry et al. (1962) and Kroening, Pond, and Loosli (1965) have shown that methionine is the most limiting amino acid in soybean meal, and have found that addition of methionine to a purified diet containing soybean meal had a significant growth promoting value. Pfander and Tribble (1955); Sewell and Keen (1958); Acker, Catron, and Hays (1959); and Chance, Mertz, and Beeson (1960) all reported no improvement in growth rate when methionine was added to the rations of weanling pigs.

Becker et al. (1955) reported that 0.25% methionine was required by

weanling pigs in rations containing 0.17% cystine. Evans (1958) reported that 0.53% methionine plus cystine probably represented the minimum requirement of weanling pigs. The most recent methionine requirements listed by the NRC (1964) are 0.85% of a 22%-protein diet for baby pigs (5 to 10 lb.) and 0.55% of an 18%-protein diet for growing pigs (25 to 75 lb.), which indicates that about 0.70% of a 22% diet should meet the requirements of 10 to 25-lb. pigs.

Influence of Energy, Protein, and Amino Acid Level on the Requirement for Certain Amino Acids

Abernathy, Sewell, and Tarpley (1958) have reported an interaction between energy level and lysine requirements in which the growth-depressing effect of added lysine was overcome by increased energy. Mitchell et al. (1965) related lysine requirements to metabolizable energy (ME) and found maximum efficiency when the rations contained 0.23% lysine per 1000 kcal ME in the diet.

Brinegar et al. (1950) reported lysine requirements of 0.6 and 1.2% when growing pigs were fed 10.6 and 22% protein rations, respectively. They showed that differences are eliminated if the amino acid requirement is expressed as a percentage of the protein present, i.e. 5.6% in their case. Pfander and Tribble (1955) have reported a lysine requirement of 5% of the total protein, and Costain and Morgan (1961), 5.8 per cent.

Methionine and cystine requirements of weanling pigs in the range of 3.2 to 3.5% of total protein have been reported by Becker et al. (1955) and Pfander and Tribble (1955).

Chance et al. (1958) and McWard et al. (1959) have both reported that, as a percentage of total protein, the lysine requirement decreases as protein increases. Acker et al. (1959) found no response to lysine when

a corn soybean meal diet contained 14% protein, whereas a 12%-protein ration responded favorably to a 0.10%-lysine addition.

Sewell and Keen (1958) did not observe any interaction of methionine and protein level when they added methionine to adjust the total content to 2% of the dietary protein from weaning to slaughter. Kroening et al. (1965) reported that the methionine plus cystine requirement decreased from 4.2% on a 12%-protein diet to 2.8% on a 25%-protein diet.

It has been shown that cystine can replace 40 to 50% of the methionine requirement in the rations of pigs (Becker et al., 1955; Evans, 1958). The NRC requirements for swine accept up to 50% of the methionine requirement as cystine (NRC, 1964).

Becker et al. (1955) reported that supplementary levels of 0.3 or 0.4% methionine inhibited growth of weanling pigs. Pfander and Tribble (1955) and Chance et al. (1960) have both reported that supplemental methionine, when added with lysine, depressed the growth of weanling pigs. These results suggest imbalance of the amino acids.

Energy and Nitrogen Digestibility and Nitrogen Retention

Lloyd and Crampton (1957) reported a highly significant inverse relationship between fatty-acid chain length and the apparent digestibilities of fats by baby pigs. Lloyd, Crampton, and MacKay (1957) further showed that age of pigs influenced apparent digestibility of energy, crude protein, and other nutrients. These workers as well as Combs et al. (1963) and Eusebio et al. (1965) have all reported an increase in digestibility of fats and protein between the 3rd and 6th or 7th week of age. Rutledge et al. (1961) and Long, Hudman, and Peo (1962) reported an increase in protein digestibility and an increase in

urinary nitrogen (i.e. decreased retention) with increasing age or weight of young pigs. Asplund et al. (1960) observed that when fat was added to rations for 8-week-old pigs, the apparent digestibility of ether extract and protein was increased. Lowrey et al. (1958) also observed an increase in protein digestibility associated with an increase in energy in the rations of young growing pigs. Kuryvial and Bowland (1962), Lowrey et al. (1963), and Robinson et al. (1964) all have reported that fat level has no consistent effect on nitrogen digestibility or retention. Likuski, Bowland, and Berg (1961) reported that retention of digested nitrogen was reduced with higher-energy, compared to lower-energy, diets.

The protein level of the diet appears to affect the digestibility of the protein in that diet, but this is confounded with an age or weight variable. Likuski et al. (1961), Kuryvial and Bowland (1962), and Robinson et al. (1964) have all reported high nitrogen digestibility and retention with high levels of dietary protein when fed to young pigs, however, the digestibility and retention fall as the pigs mature. With pigs on low protein, this decline in retention does not appear to occur.

Effect of Amino Acid Supplementation on Nitrogen Balance

Lysine supplementation has been shown to increase nitrogen digestibility (Jones, Hepburn, and Boyne, 1961; Long et al., 1962) and nitrogen retention (Jones et al., 1961; Soldevila and Meade, 1964; and Robinson, 1965).

Meade (1956) and Meade and Teter (1956) reported that supplemental lysine did not affect nitrogen retention of growing pigs, while Long et al. (1962) reported decreased nitrogen retention due to lysine supplementation of baby pig rations.

Long et al. (1962) reported that methionine supplementation increased

nitrogen digestibility and nitrogen retention on corn-soybean meal rations for baby pigs. Similar results were reported by Kroening et al. (1965) for growing pigs. Methionine was found to have no effect on nitrogen digestibility or retention when added to barley-soybean meal rations for growing pigs (Soldevila and Meade, 1964; Meade et al., 1965). Jones et al. (1961) reported that nitrogen retention, expressed as a percentage of dietary nitrogen, was depressed by the addition of methionine to 18%-protein diets composed of barley and groundnut meal.

Evans (1958) supplemented all-vegetable diets for growing pigs simultaneously with lysine and methionine and reported increased nitrogen retention, but variable results for nitrogen digestibility. He reported either no change or increased digestibility.

Serum Protein Levels

Although changes in the composition and maturation of swine serum protein associated with age and with diet have been studied quite extensively (Knill, Podleski, and Childs, 1958; Lecce and Matrone, 1960; Miller et al., 1961; and Lecce, Morgan, and Matrone, 1962), changes in the absolute levels of serum protein due to dietary protein level and protein quality in early weaned pigs have not.

Lecce and Matrone (1960) have reported lower serum protein levels in artificially reared pigs fed cows' milk, artificial milk, or amino acid milk than in pigs allowed to suckle until 2 weeks of age. Rutavist (1958) reported that piglets removed from the sow after 48 hours and placed on an artificial starting diet had lower serum protein levels at 4 and at 13 weeks than had naturally reared pigs.

Cartwright and Wintrobe (1948) reported that pigs fed 10%-protein diets had lower serum protein and serum albumin levels than did pigs fed

26%-protein diets where the protein was derived from casein. In this trial, 3-week-old weanling pigs were used but the blood samples were not taken until 5 to 6 months after the initiation of the trial.

Pond et al. (1965) found that serum protein levels were depressed when low-protein diets were fed to 3 to 5-week-old weanling pigs for 60 days. He also reported that the depression was greater when high levels of fat were included in the low-protein ration than when low levels of fat were included.

Athens, Cartwright, and Wintrobe (1958) reported that serum protein and albumin levels were depressed in 7-week-old pigs which had received a lysine-deficient, corn-cottonseed diet for 4 weeks. They also showed that the addition of a lysine antagonist further reduced the serum protein levels.

Cahilly et al. (1963) reported that a lysine deficiency was similar to a restriction in dietary protein inasmuch as it resulted in decreased serum protein and albumin levels. Brooks et al. (1964) have also studied the effect of lysine supplementation to a lysine-deficient, corn-peanut meal diet for young swine and have reported that lysine supplements resulted in increased serum protein and albumin levels. They concluded that serum albumin levels might become useful as a measure of protein adequacy.

EXPERIMENTAL

Objectives

These experiments were conducted to study fat levels and lysine and methionine supplementation of diets containing different levels of crude protein when fed to Lacombe x Yorkshire and Hampshire x Lacombe-Yorkshire pigs from 3 to 9 weeks of age. Four experiments were conducted and the criteria measured were:

- 1) Rate of gain, feed consumption, and efficiency of feed utilization during the 6-week period in Experiment 1.
- 2) Apparent nitrogen and energy digestibilities and nitrogen retention at 6 weeks of age using metabolism-cage methods in Experiment 2.
- 3) Apparent nitrogen and energy digestibilities at 9 weeks of age using Cr_2O_3 as an indicator in Experiment 3. An auxiliary experiment was conducted to determine the effects of time of feces collection and method of confinement on apparent nitrogen digestibility using Cr_2O_3 methods.
- 4) Serum protein levels at 9 weeks of age in Experiment 4.

Formulation of Experimental Rations

The formulation and composition of the rations fed are shown in Table 1. The caloric density of the rations was varied by the addition of stabilized tallow to the rations at the expense of sucrose on an equal weight-replacement basis. Protein levels were adjusted by replacing sucrose with the protein supplement on a weight basis. The ratio of soybean meal to herring meal was kept constant in all rations.

Limestone and dicalcium phosphate were added so that each ration contained the NRC (1964) requirement for calcium and phosphorus. The vitamin and zinc supplements were added at such levels that there was an

Table 1. Formulation and composition of rations

Ration no.	1A	2A	3A	4A	5A	1B	2B	3B	4B	5B	1C	2C	3C	4C	5C
Fat level (added) %	0	0	0	0	0	4	4	4	4	4	8	8	8	8	8
Protein (by analysis) %	13.0	16.4	20.8	12.6	16.6	12.6	16.0	20.7	13.0	16.5	13.0	16.7	21.1	13.1	16.6
Energy of combustion kcal/g	3.73	3.82	3.94	3.85	3.84	4.14	4.04	4.17	4.03	4.09	4.24	4.31	4.19	4.27	4.30
<u>Ingredients</u>															
Fat	0	0	0	0	0	4.0	4.0	4.0	4.0	4.0	8.0	8.0	8.0	8.0	8.0
Wheat	65.0	65.0	65.0	65.0	65.0	65.0	65.0	65.0	65.0	65.0	65.0	65.0	65.0	65.0	65.0
Protein mix ¹	7.8	14.8	21.8	6.8	14.2	7.8	14.8	21.8	6.8	14.2	7.8	14.8	21.8	6.8	14.2
Sucrose	24.0	17.5	10.9	24.0	17.5	20.0	13.5	6.9	20.0	13.5	16.0	9.5	2.9	16.0	9.5
Dicalcium phosphate	2.0	1.5	1.0	2.0	1.5	2.0	1.5	1.0	2.0	1.5	2.0	1.5	1.0	2.0	1.5
Limestone	0.25	0.25	0.35	0.25	0.25	0.25	0.25	0.35	0.25	0.25	0.25	0.25	0.35	0.25	0.25
Iodized salt	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
Zinc sulphate	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05
Vit B complex mix ²	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2
Vit B ₁₂ (9 mg/lb.)	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1
Antibiotic TM-10 ³	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1
Vitamins A and D ⁴	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
L-lysine monohydrochloride				0.7	0.4				0.7	0.4				0.7	0.4
DL-methionine (90%)				0.3	0.2				0.3	0.2				0.3	0.2
Total	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100

¹Protein mix: soybean meal (44%), 60%; herring meal (72%), 40%.

²Vit B complex mix contained the following B vitamins/kg of vitamin mix: riboflavin, 4.4 g; calcium pantothenate, 8.8 g; niacin, 19.8 g; choline chloride, 21.97 g; folic acid, 132.0 mg.

³Antibiotic: contained 22 g terramycin/kg of mix.

⁴To supply 220,000 IU of vitamin A/100 kg and 55,000 IU of vitamin D₂/100 kg of ration.

adequate margin of safety to meet requirements even if a reduction in feed intake resulted from the increased energy levels.

The three fat levels and the three protein levels were factorially arranged, but the amino acids were added as supplements to the two lower protein rations only. In this experiment, L-lysine (as L-lysine monohydrochloride) and DL-methionine were added to the 12 and 16%-protein diets to raise the concentrations of these amino acids to the same concentrations as they were calculated to be in the 20%-protein ration at each fat level. The calculated lysine, methionine, and cystine content of the rations, using average values from the literature (Hubbell, 1965), is shown in Table 2.

Table 2. Calculated lysine, methionine, and cystine composition of rations

Ration no.	Lysine %	Methionine %	Cystine %	Methionine and cystine %
1A, 1B, 1C	0.55	0.23	0.18	0.41
2A, 2B, 2C	0.83	0.32	0.24	0.56
3A, 3B, 3C	1.12	0.41	0.29	0.70
4A, 4B, 4C	1.09	0.52	0.17	0.69
5A, 5B, 5C	1.14	0.52	0.23	0.75

All rations were mixed at the University of Alberta Livestock Farm elevator and were bagged and stored in the old swine barn.

For the digestibility trials, 1% Cr_2O_3 ¹ was added to the rations listed in Table 1 and mixed in a Hobart² mixer for approximately 25 minutes.

¹Fisher Scientific Company, Fair Lawn, New Jersey. Certified reagent grade.

²Hobart Manufacturing Company Ltd., Toronto, Ontario. Model D300.

Methods and Procedures

The feeding, digestibility, and metabolism portions of the experiments were conducted during the months of May, June, July, and August, 1965. Pigs from Lacombe x Yorkshire and Hampshire x Lacombe-Yorkshire litters were weaned at 3 weeks of age. They were allowed to remain for 2 more days in the farrowing barn where they had access to the creep feed routinely used by the University of Alberta, and they were then moved to the Muttart feeding barn, were weighed, and were placed on test. The pigs were identified at birth and were treated routinely for the prevention of anemia with a 2-ml injection of Pigdex 100¹ (100 mg/ml injectable iron) at 4 days of age. Male pigs were left intact for the duration of the experiment.

Only pigs weighing 4.5 kg or over at the start of the experiment were used. There was one exception to this, in which case the pig had weighed 4.5 kg at weaning but had lost 0.45 kg before the test began.

The pigs were allotted to rations as they were weaned. Each ration was fed to a lot of four pigs. The lots were balanced with respect to breed groups and breed groups were balanced with respect to sex, i.e. there was a male and a female from each breed group in each lot. The pigs were allotted randomly within the breed group, and sex within breed group limitation, to each of the 15 diets listed in Table 1.

Experiment 1 — Growth, feed consumption, and efficiency of feed utilization data

The pigs were housed in the Muttart feeding barn at the University Livestock Farm. They were individually fed for three 1-hour periods daily starting at 7:00 am, 11:30 am, and 4:30 pm. The pigs ran together in groups of eight when not being fed. Water was supplied in automatic watering bowls and was available to the pigs except when they were being fed. The pigs were

¹Cyanamid of Canada Ltd., Montreal, Quebec.

weighed weekly and individual feed consumption was recorded.

Experiment 2 — Metabolism experiment

After the pigs had been on trial for 3 weeks, a female from breed group I and a male from breed group II were removed from the pens and were placed in rabbit cages for the metabolism trial. The cages were the same as were described by Hussar (1958) except that a wooden, removable feed trough was used and water was supplied in a small trough in the cage.

The pigs were allowed 2 days for acclimatization and were then placed on test for 2 days. The pigs were fed in the cages at the same time as they had been fed in the pens, and water was available at all times. Feed consumption was recorded for both the acclimatization and test periods. During the test period a total collection of feces and urine was made.

The feces were collected on a fiberglass screen which was placed over the urine collection tray and between the tray and the cage. The feces were found to fall through the mesh of the cage quite readily but, to insure complete collection, each pig was removed after the acclimatization period and the cages were scrubbed before the actual trial began and again after the trial was terminated.

The urine collection trays were attached beneath the cages and were placed on a sufficient slant to have all of the urine run to the funnel-shaped drain in the trays. The urine was funnelled into a plastic 1-gallon pail which contained about 50 ml of 50% H_2SO_4 . A piece of polyethylene sheeting was placed around the two sides and back of the cages to insure complete collection of urine from female pigs. Some contamination of the feces with urine was unavoidable using this system.

The feces were removed from the screen following the morning and evening feedings each day. At the same time, urine was collected from the pail and was stored in closed glass jars.

The feces were stored in plastic bags in a refrigerator during the trial. Following the completion of the trial, the feces were air-dried in a forced-air oven at 50 C for 48 hours, were allowed to equilibrate with the moisture of the air for 48 hours, and were then weighed. The fecal samples were ground in a Wiley no. 1 mill¹ to pass through a 2 mm mesh screen (#20 screen), were mixed, and a sample taken. The sample was stored in a closed plastic bag in the refrigerator (3 C) in the laboratory until analyzed.

The acidified urine was measured in a 1000-ml graduated cylinder, a sample was taken in a glass-stoppered bottle, and the sample was stored in the refrigerator at the laboratory until analyzed.

Experiment 3 — Chromium sesquioxide digestibility experiment

Three days before the end of the experiment, the pigs were fed the ration which they had previously been receiving but with 1% Cr₂O₃ added. The pigs were first given this ration at 11:30 am. Beginning at 8:00 am the following day, feces samples were collected from each pig after each meal until 28 hours after the start of the collection. The samples were stored in plastic bags in the refrigerator between collections. After the final collection, the feces were dried and stored as described for the metabolism-experiment feces.

Experiment 4 — Serum protein levels

A blood sample was obtained by syringe withdrawal from the anterior vena cava of each animal at the completion of the 6-week feeding experiment using the method of Carle and Dewhirst (1942). The blood was allowed to clot in a test tube and the serum was withdrawn by means of an eye-dropper. Some cells were drawn up in the eye-dropper so the serum was centrifuged at

¹A. H. Thomas Company, Philadelphia, Pennsylvania.

3000 rpm for 10 minutes to remove the cells. The serum was then decanted into another test tube and was stored in a freezer until analyzed for protein.

Methods of chemical analyses

The diets and feces were analyzed for gross energy content using a Parr Oxygen Bomb Calorimeter¹. Nitrogen analysis of the diets, feces, and urine were done by the Kjeldahl method for nitrogen (AOAC, 1960). A commercial "Kel-Pak"² was used to supply the required amount of catalyst, and boric acid was used to retain the ammonia. Protein level was calculated using a conversion factor of 6.25.

The method of Cr_2O_3 determination was that reported by Hill and Anderson (1958), (Appendix A). The absorption spectra (Fig. 1, Appendix A) was determined over a range of wavelengths, and a reference curve was prepared at the wavelength of maximum absorption (Fig. 2, Appendix A).

The serum protein was determined using an Auto-Analyzer³ equipped with a serum protein manifold. This method is essentially a colorimetric determination of protein by the Biuret method. Hemolysis occurred in most of the samples and resulted in varying degrees of color in the serum samples. This color affected the absorption independently of the color developed by the protein-Biuret reagent reaction. To correct for this, the optical density of a blank serum sample, without the Biuret reagent added, was determined and the optical density reading thus obtained was subtracted from the optical density of the serum-Biuret reagent sample. The serum protein level was obtained from a standard curve prepared at the same time as the serum was analyzed.

¹Parr Instrument Company, Moline, Illinois. Temperature changes registered by a Brown Electronik Recorder manufactured by Minneapolis-Honeywell Regulator Company, Philadelphia, Pennsylvania.

²Matheson Scientific, East Rutherford, New Jersey. Supplies Hg catalyst, K_2SO_4 and CuSO_4 .

³Technicon Instrument Corporation, Chauncy, New York.

Methods of statistical analyses

All weights of pigs and feed consumption were recorded in pounds and were converted to the metric system for presentation using the conversion factors, 1 lb. equals 454 g and 2.2 lb. equal 1 kilogram. Rate of gain and feed consumption were analyzed using the pound figures and the mean squares obtained have been divided by a factor of 4.84 (2.2^2) to convert them to the metric system.

All of the data were analyzed statistically by analysis of variance. The analysis was done on an IBM 7040 computer using the library program BMD02V (University of Alberta, 1965a). An analysis of covariance was also done on the growth data using initial weight as the covariate. This was done on the IBM 7040 computer using the library program BMD03V (University of Alberta, 1965b).

Analysis of covariance of average daily gain, average daily feed intake, and efficiency of feed utilization data using initial weight on test as the covariate showed the same factors to be significant as were determined by the analysis of variance. Therefore, only results of analysis of variance are discussed.

The data were analyzed as a 3 x 5 x 2 x 2 factorial in which the factors were fat, protein treatment (which included both protein level and amino acid supplement), breed group, and sex. This method did not permit obtaining a simple measure of the variance due to protein or amino acid main effects or fat x protein or protein x amino acid interactions. The significance of the variance due to protein main effects and fat x protein interactions was determined by repeating the analysis of variance, but data relating to amino acid-supplemented rations were deleted. The "F" values relating to amino acid main effects and protein x amino acid interactions

were obtained by the method of mean separation by the functional analysis of variance using orthogonal comparisons (LeClerc, 1957).

Missing data were replaced by the average value of the group from which the data were missing. This was considered to be a better estimate than any which could be arrived at by use of a missing value formula¹. Data for four pigs were missing for the apparent digestible nitrogen (ADN) and for five pigs for the apparent digestible energy (ADE) determinations in Experiment 3, and for three pigs for serum protein levels in Experiment 4.

A composite error term was used in all analysis of variance in which missing data were estimated. The sums of squares for all third-order interactions and residual was used to compute the composite error. This was done so that the error degrees of freedom would not be reduced by too large a percentage and important results overlooked.

Differences between means were obtained by using Duncan's new multiple range test. Steel and Torrie (1960) state that this test can be used regardless of the significance of the "F" test.

¹Dr. R. T. Hardin, personal communication.

RESULTS AND DISCUSSION

Experiment 1 — Daily Gain, Daily Feed, and Efficiency of Feed Utilization

The means for average daily gain, average daily feed intake, and efficiency of feed utilization are given in Table 3, and the mean squares obtained by analysis of variance of the data are given in Table 4.

Daily gain

Protein treatment exerted a significant effect upon average daily gain. The effect was due to both protein levels and amino acid supplementation. Each increment of protein resulted in a significant increase in average daily gain. The increase which resulted when the protein level was raised from 16 to 20% was not as great as that which occurred when the protein was raised from 12 to 16 per cent. The average daily gains for the 12, 16, and 20%-protein rations were 0.11, 0.22, and 0.28 kg/day, respectively. These results agree with those of Peo et al. (1957), Abernathy et al. (1958), and Aunan et al. (1961), all of whom reported increased gains in young pigs when protein levels were increased to 20% or higher.

Lysine and methionine supplementation appeared to have more effect on the 16%-protein ration than it did on the 12%-protein ration. The average daily gain on the 12%-protein ration with added lysine and methionine was 27% higher than it was on the unsupplemented 12%-protein ration (0.14 vs. 0.11 kg/day), while the supplemented 16%-protein ration supported gains which were 36% higher than the unsupplemented ration containing 16% protein (0.30 vs. 0.22 kg/day). Duncan's multiple range test (at $P < 0.01$) shows that the increase due to the addition of lysine and methionine to the 12%-protein ration was not enough to make it significantly different

Table 3. Experiment 1 — Daily gain, feed consumption, and efficiency of feed utilization means

Ration	Fat %	Protein %	Amino acid ¹	No. of pigs	Daily gain ² kg	Daily feed intake ² kg	Feed/ kg gain ² kg
1A	0	12		4	.10	.33	3.18
2A	0	16		4	.21	.45	2.16
3A	0	20		4	.26	.50	1.86
4A	0	12	+	4	.17	.39	2.27
5A	0	16	+	4	.30	.55	1.80
1B	4	12		4	.11	.31	2.90
2B	4	16		4	.22	.46	2.12
3B	4	20		4	.31	.53	1.72
4B	4	12	+	4	.13	.34	2.65
5B	4	16	+	4	.28	.48	1.76
1C	8	12		4	.11	.28	2.51
2C	8	16		4	.22	.46	2.07
3C	8	20		4	.27	.48	1.77
4C	8	12	+	4	.13	.33	2.57
5C	8	16	+	4	.31	.52	1.69
Factor means							
Fat	0			20	.21	.44	2.30
	4			20	.21	.43	2.27
	8			20	.21	.41	2.14
Protein		12		12	.11 ^a	.31 ^a	2.97 ^a
treatment		16		12	.22 ^b	.46 ^b	2.14 ^{bc}
		20		12	.28 ^c	.50 ^{bc}	1.79 ^{bc}
		12	+	12	.14 ^a	.35 ^a	2.51 ^{ab}
		16	+	12	.30 ^c	.52 ^c	1.77 ^c
Breed group:							
Lac x York				30	.21	.42	2.21
Hamp x Lac-York				30	.21	.43	2.26
Sex:							
Male				30	.20	.42	2.26
Female				30	.22	.44	2.21

¹ + signifies that lysine and methionine were added to the rations so that the concentration of these amino acids would be the same as in the 20% ration.

² Any means in a group of factor means having the same superscript are not significantly ($P < 0.05$) different from each other.

Table 3A. Breed group and sex effect on average daily feed intake (g)

Breed group	Sex		Avg
	Male	Female	
Lac x York	.87	.98	.93
Hamp x Lac-York	.96	.94	.95
Avg	.92	.96	

Table 4. Mean squares obtained by analysis of variance of growth data

	df	Average daily gain	Average daily feed	Feed/kg gain
Fat level	2	.00003	.00372	.1302
Breed group (BG)	1	.00012	.00174	.0346
Sex	1	.00455	.00610	.0427
Protein treatment (PT)	4	.08364**	.10510**	3.1289*
- Amino acid level ¹	(1)	.03926**	.03399**	2.07*
- Protein x amino acid level ¹	(1)	.00578	.00066	.02
Fat level x BG	2	.00244	.00085	.4496
Fat level x sex	2	.00165	.00333	.1290
Fat level x PT	8	.00160	.00252	.2007
BG x sex	1	.00104	.01504*	.0792
BG x PT	4	.00205	.00913	.0165
Sex x PT	4	.00062	.00157	.1449
Error	8	.00235	.00245	.2924
Total df		59	59	59
Protein level	2	.09423**	.12638**	4.3972**
Fat x protein	4	.00087	.00177	.1952
Error	4	.00244	.00171	.3716

¹ Amino acid level and protein x amino acid level means are comparisons from within the protein treatment factor.

* Significant at $P < 0.05$.

** Significant at $P < 0.01$.

from the unsupplemented 12%-protein ration. The increase which occurred when the amino acids were added to the 16%-protein ration was enough to make a significant difference between the supplemented and unsupplemented rations. Meade et al. (1965) have reported increased gains when lysine and methionine were added to a 16%-starter diet.

These results are in agreement with expected results when reference is made to NAS — NRC "Nutrient Requirements for Swine" (NRC, 1964). They recommend 22%-protein diets for pigs 10 to 25 lb., suggesting that gain should increase as protein is increased up to that level. Requirements of 1.07% lysine and 0.70% methionine plus cystine are suggested in this publication. Table 2, p. 15, shows the calculated lysine and methionine plus cystine contents of the diets. It can be seen that the unsupplemented diets contained less of these amino acids than is recommended, while the supplemented diets contained very nearly the recommended levels.

Fat levels had no effect on average daily gain, which agrees with the findings of Asplund et al. (1960), Lowrey et al. (1962), and Eusebio et al. (1965).

Breed group and sex had no effect on daily gain and there were no significant interactions.

Daily feed intake

Protein content of the ration was found to affect average daily feed intake. Analysis by Duncan's multiple range test showed that the pigs receiving rations with 12% protein or 12% protein with added amino acids consumed significantly ($P < 0.01$) less feed per day than did the other pigs. Amino acid supplementation was also found to have an effect on feed intake with significantly ($P < 0.01$) more of the supplemented rations being consumed than the comparable unsupplemented rations. The average daily feed

intake for the 12, 16, and 20%-protein rations was 0.31, 0.46, and 0.50 kg per day and for the amino acid-supplemented 12 and 16%-protein rations was 0.35 and 0.52 kg /day, respectively.

Bowland (1964a) had previously reported that, for pigs 3 to 10 weeks old, feed and thus energy consumption is positively associated with the protein level of the ration if the protein level falls below approximately 14 per cent. In this experiment, this relationship appeared to extend to 16% protein but levels between 12 and 16% protein were not tested.

Fat level in the rations did not significantly affect feed intake.

There was a significant ($P < 0.05$) sex x breed group interaction which showed that the Lacombe x Yorkshire females consumed more feed than did the males in that breed group, while the Hampshire x Lacombe-Yorkshire males consumed more than did the females. Table 3A shows the interaction means.

Efficiency of feed utilization

Protein was found to influence the efficiency of feed utilization. Using mean separation, this was found to be due to both protein levels and amino acid levels. Efficiency of feed utilization was significantly improved ($P < 0.05$) as protein was raised from 12 to 16% in the ration (2.97 vs. 2.14 kg feed/kg gain); however, when protein was raised to 20%, the further increase was not enough to be significant. These results agree, in general, with those reported by Peo et al. (1957) and Lloyd and Crampton (1961) who reported that feed efficiency improved when protein levels were increased to 20% or higher for young pigs.

The amino acid-supplemented rations were used more efficiently ($P < 0.01$) than were the unsupplemented rations containing the same levels of protein. The effect of supplementing with amino acids was to reduce

the amount of feed required per unit of gain; about 15% on the 12%-protein diets (2.97 vs. 2.51 kg feed/kg gain unsupplemented vs. supplemented 12% protein, respectively) and 17% on the 16% diet (2.13 vs. 1.76 kg feed/kg gain). These results agree with those of Evans (1958) and Meade et al. (1965) who reported increased feed efficiency when lysine and methionine were added to pigs' diets.

Other treatment effects did not influence efficiency of feed utilization.

Experiment 2 — Metabolism Experiment: Energy and Nitrogen Digestibility, and Nitrogen Retention of 6-Week-Old Pigs

The data for ADE, ADN, and nitrogen retention, and the analysis of these data, are given in Tables 5 and 6.

Energy digestibility

With the exception of two pigs, the ADE coefficients determined appeared quite uniform for all of the pigs. None of the treatment factors affected energy digestibility when 0.05 or less was used as the probability level for significance. Protein treatment was found to exert an effect at the probability level of 0.10. Analysis of the protein-treatment means by Duncan's multiple range test ($P < 0.05$) did show that, when the 12%-protein diet was supplemented with lysine and methionine, the ADE was significantly higher than in the 16%-protein ration. The ADE coefficients obtained on the 12, 16, and 20%-protein rations were 89.2, 86.6, and 88.7%, respectively; and on the amino acid-supplemented rations were 91.4 and 89.6% on the 12 and 16% rations.

The result obtained by Duncan's multiple range test could quite reasonably be attributed to chance, as it was found that the two pigs having the lowest ADE were in the 16%-protein group and these were considerably below the rest of the ADE values obtained on the 16%-protein ration or on any of the other rations. If these two values are removed, the average ADE coefficient of the remaining four pigs on the 16%-protein ration is 88.8% which is intermediate between the 20%-protein and the 12%-protein ADE means which were 88.7 and 89.2%, respectively.

Nitrogen digestibility

The ADN coefficients obtained in this experiment showed considerably more variation than did the ADE coefficients obtained at the same time.

Table 5. Experiment 2 — Metabolism trial: energy and nitrogen digestibilities and nitrogen retention means

Ration	Fat %	Protein %	Amino acid ¹	No. of pigs	Digestibility		Retention	
					Energy ² %	Nitrogen ² %	Gross nitrogen ² %	ADN ² %
1A	0	12		2	88.5	80.6	40.9	46.5
2A	0	16		2	85.9	79.6	43.2	53.3
3A	0	20		2	88.3	86.9	56.9	65.5
4A	0	12	+	2	92.5	88.0	59.0	66.9
5A	0	16	+	2	88.1	83.1	58.5	70.4
1B	4	12		2	91.5	85.6	53.1	61.8
2B	4	16		2	89.6	86.3	54.6	63.3
3B	4	20		2	88.9	86.8	58.2	67.1
4B	4	12	+	2	90.0	83.8	55.5	66.3
5B	4	16	+	2	88.7	86.4	61.5	71.2
1C	8	12		2	87.4	80.2	38.4	47.7
2C	8	16		2	84.1	80.2	41.5	51.5
3C	8	20		2	89.0	88.0	52.4	59.6
4C	8	12	+	2	91.7	88.0	65.8	74.7
5C	8	16	+	2	91.9	89.6	57.5	64.1
Factor means								
Fat	0			10	88.6	83.6	51.7	60.5
	4			10	89.8	85.8	56.6	65.9
	8			10	88.8	85.2	51.1	59.5
Protein		12		6	89.2 ^{ab}	82.1 ^a	44.1 ^a	52.0 ^a
treatment		16		6	86.6 ^a	82.0 ^a	46.5 ^{ab}	56.0 ^{ab}
		20		6	88.7 ^{ab}	87.2 ^b	55.8 ^{ab}	64.0 ^{ab}
		12	+	6	91.4 ^b	86.6 ^{ab}	60.1 ^b	69.3 ^b
		16	+	6	89.6 ^{ab}	86.4 ^{ab}	59.2 ^b	68.6 ^b
Breed group - sex³:								
Lac x York - males				15	89.2	84.8	53.1	62.2
Hamp x Lac-York - females				15	89.0	85.0	53.2	61.8

¹ + signifies that lysine and methionine were added to the rations so that the concentration of these amino acids would be the same as in the 20% ration.

² Any means in a group of factor means having the same superscript are not significantly ($P < 0.05$) different from each other.

³ Breed group and sex were confounded in this experiment.

Table 6. Mean squares obtained by analysis of variance of metabolism trial data

	df	Digestibility		Retention	
		Energy	Nitrogen	Energy	Nitrogen
Total df		29	29	29	29
Fat level	2	3.55	12.10	90.06	118.77
Breed group - Sex ¹ (BG-S)	1	0.41	0.32	0.07	1.11
Protein treatment (PT)	4	18.29	39.38	326.54	354.59
- Amino acid level ²	(1)	1.32	116.68**	1231.95**	1336.08**
- Protein x amino acid level ²	(1)	0.19	0.05	16.58	34.39
Fat level x BG-S	2	7.45	22.35	99.66	69.78
Fat level x PT	8	8.16	16.94	54.10	53.09
PT x BG-S	4	1.56	7.60	40.69	35.33
Error	8	6.48	10.52	97.24	111.62

¹Breed group and sex are confounded inasmuch as a male from breed group I and a female from breed group II were tested.

²Amino acid level and protein x amino acid level means are comparisons from within the protein treatment factor.

**Significant at $P < 0.01$.

The ADN coefficients ranged from 74 to 92 per cent. Analysis of variance showed that the protein treatment factor exerted an effect on ADN when the probability level was reduced to 0.10. Analysis by Duncan's multiple range test ($P < 0.05$) shows that there was a difference between the 20%-protein ration and the 12 and 16%-protein rations with ADN being highest on the 20% ration (82.1, 82.0, and 87.2% were the ADN coefficients found on the 12, 16, and 20% protein rations, respectively). Kuryvial (1961) found no significant difference in ADN of rations due to protein levels when significance was measured at the 0.05 probability level. Likuski (1959) has reported that increasing protein to 18 from 14% resulted in increased ADN coefficients.

Comparisons of the means of the supplemented with the corresponding unsupplemented rations showed a highly significant increase in ADN coefficients with the added amino acids. The ADN coefficients of the supplemented 12 and 16%-protein rations were 86.6 and 86.4%, and the values obtained with the unsupplemented rations were 82.1 and 82.0%, respectively. Evans (1960) has published tabular results which showed about the same increases in ADN coefficients on an all-vegetable diet as were obtained here when lysine and methionine were supplemented.

All other treatment factors and factor interactions were found to have no effect on ADN coefficients. Kuryvial (1961) also reported no effect of fat level on ADN coefficients of baby pigs when feeding levels of up to 30% fat.

Nitrogen retention

The treatment effects were found to be similar for nitrogen retention expressed either as a percentage of gross nitrogen consumed or as a percentage of the ADN.

Analysis by Duncan's multiple range test ($P < 0.05$) of the protein treatment means shows no statistical difference in protein retention due to protein level, although there was an apparent increase in nitrogen retained as the protein level increased. Nitrogen retention expressed as a percentage of gross nitrogen ingested was found to be 44.1, 46.5, and 55.8% with the 12, 16, and 20%-protein rations, respectively. Kuryvial and Bowland (1962) also reported a nonsignificant increase in nitrogen retention as protein level was increased from 14 to 18 per cent.

Mean comparisons of the amino acid-supplemented diets and the unsupplemented diets of the same protein level show that the percentage of nitrogen retained was increased ($P < 0.01$) by adding lysine and methionine to the 12 and 16%-protein rations. The nitrogen retention values obtained with the 12 and 16%-protein rations supplemented with amino acids were found to be 60.1 and 59.2% and with the unsupplemented rations, 44.1 and 46.5%, respectively. This is expressing nitrogen retention as a percentage of gross nitrogen ingested.

The increased nitrogen retention due to amino acid supplementation was what was expected on the basis of the increased rate of gain and improved efficiency of feed utilization with amino acid supplementation. It seems evident that either one or both of the amino acids supplemented were limiting in the unsupplemented rations, and by adding the amino acids it was possible for the tissues to utilize more of the total nitrogen for protein anabolism. These results support the findings of Evans (1958) and Long et al. (1962), who reported that nitrogen retention was increased by supplementing lysine and methionine to pig rations.

Other treatment factors did not appear to affect nitrogen retention.

Experiment 3 — Chromium Sesquioxide Digestion Experiment: Apparent Digestible Energy and Apparent Digestible Nitrogen of 9-Week-Old Pigs

The ADE and the ADN mean coefficients obtained in the Cr_2O_3 experiment are shown in Table 7, and the mean squares obtained by analysis of variance are shown in Table 8.

Apparent digestible energy

Apparent digestible energy was not significantly influenced by protein treatment or fat level in the diet or by the sex or breed group of 9-week-old pigs. The coefficients obtained were 73.8, 75.2, and 75.4% in the 0, 4, and 8%-fat rations, respectively. The average values ranged from 72 to 77% over the five protein treatments.

When the ADE coefficients obtained by total collection methods at 6 weeks of age are compared to the ADE coefficients obtained by Cr_2O_3 indicator techniques at 9 weeks of age, the latter method showed substantially lower values (89.1 vs. 74.8% are the overall means obtained by the two methods). Reports in the literature suggest that between 6 and 9 weeks of age, the ability of pigs to digest ration nutrients may increase (Lloyd et al., 1957; Combs et al., 1963; Eusebio et al., 1965). Lowrey et al. (1962) and Takahashi and Morimoto (1963) have reported that digestibility of nutrients may be lower using the Cr_2O_3 method than those obtained by the conventional method.

Apparent digestible nitrogen

Protein treatment was found to influence the ADN in the diet. Analysis of variance by Duncan's multiple range test showed that the ADN of the 12% diet was significantly lower than was the ADN of the 20% diet.

The addition of lysine and methionine to the 12 and 16% diets did not affect the ADN of the diets significantly, but it did result in a protein x amino acid interaction which was significant. Table 7A shows interaction means.

Table 7. Experiment 3 — Chromium sesquioxide digestibility experiment: apparent energy and nitrogen digestibility means

Ration	Fat %	Protein %	Amino acid ¹	No. of pigs	ADE %	ADN ² %
1A	0	12		4	74.4	59.4
2A	0	16		4	75.2	61.7
3A	0	20		4	70.1	65.2
4A	0	12	+	4	70.4	49.0
5A	0	16	+	4	78.8	70.2
1B	4	12		4	76.9	61.4
2B	4	16		4	73.9	65.4
3B	4	20		4	77.9	73.9
4B	4	12	+	4	70.4	54.4
5B	4	16	+	4	77.0	68.8
1C	8	12		4	74.6	61.1
2C	8	16		4	76.9	70.0
3C	8	20		4	74.9	73.6
4C	8	12	+	4	73.8	58.9
5C	8	16	+	4	76.5	71.0
<u>Factor means</u>						
Fat	0			20	73.8	61.1 ^a
	4			20	75.2	64.8 ^{ab}
	8			20	75.4	66.9 ^b
Protein		12		12	75.3	60.6 ^{ab}
treatment		16		12	75.3	65.7 ^{bc}
		20		12	74.3	70.9 ^c
		12	+	12	71.6	54.1 ^a
		16	+	12	77.4	70.0 ^{bc}
Breed group:						
Lac x York				30	74.3	64.0
Hamp x Lac-York				30	75.3	64.5
Sex:						
Male				30	75.3	64.5
Female				30	74.3	64.0

¹+ signifies that lysine and methionine were added to the rations so that the concentration of these amino acids would be the same as in the 20% ration.

²Any means in a group of factor means having the same superscript are not significantly ($P < 0.05$) different from each other.

Table 7A. Protein and amino acid level effect on apparent digestible nitrogen (%) — Experiment 3

Protein	Amino acid	
	-	+
12	60.6	54.1
16	65.7	70.0

Table 8. Mean squares obtained by analysis of variance of chromium sesquioxide digestibility data

	df	ADE	ADN
Fat level	2	15.08	173.80*
Breed group (BG)	1	15.88	3.37
Sex	1	16.21	3.85
Protein treatment (PT)	4	54.71	587.51**
- Amino acid level ¹	(1)		15.13
- Protein x amino acid level ¹	(1)		350.51**
Fat level x BG	2	22.49	26.29
Fat level x sex	2	10.31	33.93
Fat level x PT	8	21.23	25.52
BG x sex	1	39.25	94.35
BG x PT	4	33.10	44.65
Sex x PT	4	5.60	14.40
Error		22.71	42.38
Error df		25	26

¹ Amino acid level and protein x amino acid level means are comparisons from within the protein treatment factor.

* Significant at $P < 0.05$.

** Significant at $P < 0.01$.

It was observed that the amino acid supplementation decreased ADN in the 12% ration (from 60.6 to 54.1%) and increased ADN in the 16% ration (from 65.7 to 70.0%), but neither of these was a significant change in itself.

It is possible that the effect of amino acid supplementation on ADN is decreased in this trial compared to the 6 week metabolism trial because an amino acid imbalance was produced. With the increasing weight of the pigs, their lysine and methionine requirement decreases and as proportionately more of the amino acids were added to the 12% diet it may, in fact, be more imbalanced than the 16% diet.

Harper (1964) has described an amino acid imbalance as a change in the proportions of amino acids in a diet which result in a depression in food intake or in growth rate that can be completely prevented by a supplement of the indispensable (i.e. essential) amino acid present in least amount in the diet in relation to the amount required for optimal performance. Amino acid imbalances may be of two types. One type results from the addition of a quantity of a protein or an amino acid mixture lacking one essential amino acid to a diet containing a moderate amount of protein. The other type results from the addition of a small amount of amino acid or acids to a diet that is low in protein. The amino acid causing the imbalance in this case is usually the one which is second most limiting for growth.

There was a significant ($P < 0.05$) increase in ADN as the fat level was increased. The ADN coefficients for the 0, 4, and 8%-fat rations were found to be 61.1, 64.8, and 66.9%, respectively. Analysis of the means by Duncan's multiple range test shows that the ADN coefficient for the 8%-fat diets was significantly higher than the ADN coefficient for the 0%-fat diets.

These results are in agreement with those of Asplund et al. (1960) and Lowrey et al. (1958). Lowrey (1962) reported that his earlier results showing an increase in ADN with increasing fat was due not to a real increase in apparent digestibility but rather due to the method of Cr_2O_3 determination which he had used. The perchloric acid digestion procedure which he first used was the method used by Asplund (1960), and it was also used in this experiment. Lowrey reported that when Cr_2O_3 was determined using the sodium peroxide fusion method he found no differences in ADN due to fat level.

Chromium sesquioxide-procedure experiment

The protein digestibility coefficients found in this trial, like the energy digestibility coefficients, were lower than were expected. The Cr_2O_3 analysis was repeated and found to agree with previous analysis. Random samples were again analyzed for protein and these analyses showed the same results as had been previously obtained. Consideration was therefore given to factors that might have influenced the results. It was thought that perhaps not enough time had been allowed between the first meal of Cr_2O_3 feed and the first collection of feces to permit equilibration of the Cr_2O_3 in the feces. The pigs had been bedded in straw in their pens and it was thought that this might dilute the Cr_2O_3 concentration in the feces to an unknown extent and, at the same time, increase the metabolic fecal nitrogen.

To test the procedure used in this Cr_2O_3 digestion experiment, a trial using 12 pigs of similar age to those used in the previous experiment was conducted in January, 1966. The pigs in this trial were either Yorkshire x Lacombe-Yorkshire or Yorkshire x Landrace-Yorkshire crosses. The sexes were equalized in both the pens and the cages. In this trial, six pigs were kept in pens and handled similarly to the previous trial. Another

six pigs were put into metabolism cages without bedding. The pig metabolism cages were the same as those described by Hussar (1958).

Following a 24-hour acclimatization period, the pigs were given feed containing 1% Cr_2O_3 and 20 hours following that the first feces sample was collected. Feces samples were then collected three times a day for two days. The seven samples from each pig were pooled and analyzed as in the previous trial. Following the next meal a new series of samples were started and this collection was also for 48 hours. A total collection of fecal production was also obtained from each of the six pigs in the metabolism cages during each of the two sampling periods.

The results obtained from this trial are listed in Table 9, and the analysis of the data in Table 10.

The results showed that there was virtually no difference between the digestibilities obtained in the pens and in the cages which indicates that straw was not being consumed to any significant amount. The ADN coefficients obtained with the pigs in the pens were 82.8 and 86.0% for the 20 and 72-hour periods, and with the pigs in the cages the ADN values were found to be 82.6 and 86.8% for the 20 and 72-hour periods, respectively. When comparing ADN coefficients obtained by Cr_2O_3 sampling with those obtained by total collection, no differences were found in either period. Apparent digestible nitrogen coefficients of 85.7 and 86.3% were obtained when total collection of feces was begun at 20 and 72 hours after the first Cr_2O_3 meal. The pigs in the pens were not included in this comparison as total collections were not made for these pigs.

When ADN values obtained by Cr_2O_3 sampling were compared for the two time periods, it was found that significantly lower ADN values were obtained when feces samples were collected 20 hours after the start of Cr_2O_3 feeding than if they were collected 72 hours after the start of Cr_2O_3 feeding.

Table 9. Chromium sesquioxide methods trial: apparent digestible nitrogen coefficients

Time, hr	20		72	
	Sample	Total collection	Sample	Total collection
Pens	84.3		86.8	
	84.0		85.7	
	80.0		86.2	
	79.7		85.4	
	84.3		87.6	
	84.3		84.6	
Avg	82.8		86.0	
Metabolism cages	86.8	86.9	87.6	87.6
	73.8	82.2	86.2	87.5
	77.6	87.3	84.6	82.1
	84.6	85.1	87.6	86.1
	84.9	85.3	88.1	87.0
	88.4	87.3	87.0	87.2
Avg	82.6	85.7	86.8	86.3

Table 10. Mean squares obtained by analysis of variance of chromium sesquioxide methods trial data (Table 9 data)

	df	Mean square
Pens vs. cages	1	0.68
Time	1	82.81**
Pens vs. cages x time	1	84.83**
Error	20	5.94
Total	23	
Sampling method	1	9.02
Time	1	33.96
Sampling method x time	1	62.62
Error	20	84.29
Total	23	

** Significant at $P < 0.01$.

The ADN coefficients for the 20-hour period were only 3 to 5 percentage units lower than they were for the 72-hour period (approximately 83 vs. 87%, respectively) which does not fully explain the greatly reduced ADN coefficients which were obtained in Experiment 3. It is believed that a reduction in ADN actually did occur between 6 and 9 weeks of age as is shown by a comparison of the values listed in Table 5, p. 28 and Table 7, p. 33. The magnitude of this reduction may not be as great as is shown because of the early collection of Cr_2O_3 feces.

The persistency of the amino acid effects may indicate that they are real effects and not due to any error in the method of determination. Lowrey et al. (1962) stated that the same relative differences in apparent digestibility values were obtained by the total collection method and the Cr_2O_3 method, but the absolute values were lower for the latter method.

The protein digestibility coefficients may have been lowered because of the high level of sucrose which was included in the ration. Skipitaris, Warner, and Loosli (1957) reported that the addition of 16% sucrose to a barley ration for market-weight pigs reduced protein digestibility by about 5 per cent. In the rations used in this trial, up to 25% sucrose was included in some rations, and the ADN coefficients increased as the sucrose decreased in all rations.

Experiment 4 — Serum Protein Levels

The data for serum protein analysis of blood obtained from each pig at the end of the trial are shown in Table 11.

Analysis of the data showed that protein treatment did not affect serum protein levels (Table 12). The addition of amino acids did appear to reduce the serum protein levels of pigs receiving the amino acid-supplemented diets compared to pigs receiving similar protein-level diets unsupplemented, but

Table 11. Experiment 4 — Serum protein level means

Ration	Fat %	Protein %	Amino acid ¹	No. of pigs	Serum protein g/100 ml
1A	0	12		4	5.66
2A	0	16		4	5.24
3A	0	20		4	6.17
4A	0	12	+	4	4.47
5A	0	16	+	4	5.43
1B	4	12		4	4.85
2B	4	16		4	5.19
3B	4	20		4	5.08
4B	4	12	+	4	4.63
5B	4	16	+	4	5.17
1C	8	12		4	4.90
2C	8	16		4	5.08
3C	8	20		4	5.05
4C	8	12	+	4	4.59
5C	8	16	+	4	4.73
<u>Factor means</u>					
Fat	0			20	5.40
	4			20	4.98
	8			20	4.87
Protein		12		12	5.14
treatment		16		12	5.17
		20		12	5.44
		12	+	12	4.56
		16	+	12	5.11
Breed group:					
Lac x York				30	4.99
Hamp x Lac-York				30	5.18
Sex:					
Male				30	5.06
Female				30	5.10

¹ + signifies that lysine and methionine were added to the rations so that the concentration of these amino acids would be the same as in the 20% ration.

Table 11A. Sex and fat level effect on serum proteins (g/100 ml)

Fat level, %	Sex		Avg
	Male	Female	
0	5.02	5.77	5.40
4	5.18	4.79	4.99
8	5.00	4.74	4.87
Avg	5.07	5.10	5.08

Table 12. Mean squares obtained by analysis of variance of serum protein data

	df	Mean square
Fat level	2	1.53
Breed group (BG)	1	0.50
Sex	1	0.02
Protein treatment (PT)	4	1.22
Fat level x BG	2	0.37
Fat level x sex	2	1.93*
Fat level x PT	8	0.37
BG x sex	1	0.04
BG x PT	4	0.90
Sex x PT	4	0.39
Error	27	0.53

*Significant at $P < 0.05$.

this was not significant. The serum protein levels for the pigs receiving the 12%-protein ration were 5.14 and 4.56 g/100 ml on the unsupplemented and supplemented diets, and for the pigs receiving the unsupplemented and supplemented 16%-protein ration were 5.17 and 5.11 g/100 ml, respectively. There was also an apparent but nonsignificant increase in serum protein levels with increasing dietary protein. The levels found were 5.14, 5.17, and 5.44 g/100 ml for the 12, 16, and 20%-protein rations, respectively. Cartwright and Wintrobe (1948) and Pond et al. (1965) both reported significant increases in serum protein levels with increased dietary protein, but they were both comparing wider protein ranges than were used in this trial.

Added fat in the ration reduced serum protein levels (significant at a probability level of 0.10) and there was also a significant fat x sex interaction (Table 11A shows interaction means). The serum protein level means for the 0, 4, and 8%-fat rations were found to be 5.40, 4.98, and 4.87 g/100 ml, respectively. Pond et al. (1965) reported decreased serum protein levels in pigs fed high levels of corn oil. They further reported higher fat concentrations in the livers of the pigs fed high fat diets and suggest this may be associated with a greater degree of necrosis and cirrhosis of the livers. This may explain the lower serum protein levels in pigs fed added fat, as most of the serum proteins are synthesized in the liver.

The interaction was associated with decreased serum protein levels in the female pigs when fat was added, while there was no change in the male pigs. Serum protein levels for the female pigs were 5.77, 4.79, and 4.74 g/100 ml for the pigs fed 0, 4, and 8%-fat rations, respectively. For the male pigs the comparable values were 5.02, 5.18, and 5.00 g/100 milliliters.

The physiological basis for this interaction might be a hormonal one.

Mallov (1958) reported that androgens (testosterone) exerted a lipotropic action on ethanol-induced fatty infiltration of the livers in rats, while estrogens did not influence the process. Brooks et al. (1964) reported no difference in serum protein levels between sexes of growing pigs fed rations which contained no added fat.

Serum proteins function as nutrient transfer factors in the blood, as amino acid sources for protein synthesis, and as immunological factors. Their level in the blood may be of considerable importance and could affect growth rate and productive efficiency. The effect of adding androgens to high-fat diets for young gilts and barrows may be beneficial, but this has not been extensively studied although the effect of testosterone in low-fat finishing rations has been reported (Beeson et al., 1955; Day et al., 1960; Beacom, 1963).

GENERAL SUMMARY AND CONCLUSIONS

Growth Data

The results indicated that the protein and amino acid level in the rations exerted more influence upon the productive efficiency of 3 to 9-week old pigs than did the energy level. Pigs fed supplemental fat showed a tendency for decreased feed intake (0.44, 0.43, and 0.41 kg/day on the 0, 4, and 8%-fat rations) and improved efficiency of feed utilization (2.30, 2.27, and 2.14 kg feed/kg gain), but these differences did not approach significance.

Increasing protein from 12 to 16 to 20% in the ration resulted in daily gains of 0.11, 0.22, and 0.28 kg/day. Supplementing the 12 and 16%-protein rations resulted in a 27 and 36% increase in gains, respectively, over the unsupplemented ration. Increasing protein from 12 to 16% resulted in significant ($P < 0.05$) improvements in feed consumption and efficiency of feed utilization, but the improvements which occurred when protein was raised from 16 to 20% was not significant. The addition of lysine and methionine to the 12 and 16% rations also favorably affected the feed consumption and efficiency of feed utilization. The response to the low levels of added lysine and methionine (0.4 and 0.2%) on the 16% diet appeared to be more effective than the higher levels (0.7 and 0.3%) which were added to the 12% diet. For example, a 36% increase in average daily gain resulted from the supplementation of the 16%-protein ration while the supplementation of the 12% ration gave an increase of 27 per cent. Similarly, the feed efficiency was improved by 17 and 15% when the amino acids were added to the 16 and 12%-protein rations, respectively. The smaller improvement brought about by lysine and methionine supplementation may be due to the fact that another amino acid was limiting (e.g. tryptophan) or it may be

that protein per se was limiting.

Because of the lack of fat level effects and the persistency of protein treatment effects, it may be fair to conclude that the feed and energy intake of baby pigs is not as dependent upon energy level in the diet as it is with some other species. Moreover, baby pigs appear capable of utilizing a wider range of energy and energy:protein ratios while eating to meet their protein requirements than is the case with some other species.

Metabolism Experiment

The results obtained on the metabolism trial, when the pigs were 6 weeks of age, substantiate the results obtained with the growth trial. The protein effects on ADN and nitrogen retention were not as pronounced as they appeared to be on growth rate and feed efficiency. The increase in ADN and nitrogen retention when lysine and methionine were added suggests that the addition of these amino acids resulted in an improved amino acid balance. This indicates that protein savings can be obtained by feeding well-balanced proteins.

Apparent digestible energy coefficients were not found to be influenced by fat levels, protein levels, or amino acid levels in the rations.

Digestibility Experiment

Energy and nitrogen digestibility coefficients obtained by the use of the Cr_2O_3 indicator technique are reported for 9-week-old pigs. Because fecal samples may have been collected before the Cr_2O_3 had sufficient time to reach maximum concentrations in the feces, the values determined are probably lower than they should be.

Apparent digestible nitrogen was influenced by protein level, being

higher in 20% (70.9% ADN) protein rations than it was in 12 (60.6% ADN) or 16%- (65.7% ADN) protein rations. A protein x amino acid interaction occurred. This may be associated with a decrease in the pigs' requirement for either lysine or methionine, or both, so that the addition of lysine and methionine to these rations no longer represented an improvement in amino acid balance. Apparent digestible nitrogen was actually decreased when the lysine and methionine were added to the 12%-protein ration. The ADN coefficients obtained were 60.6 and 54.1% for the unsupplemented and supplemented rations, respectively. These results suggest an amino acid imbalance in the ration. Apparent digestible nitrogen increased as the fat levels increased in the ration.

As with 6-week-old pigs, apparent digestible energy was not affected by any of the treatment factors.

Serum Protein Analysis

Fat, protein, or amino acid levels did not affect serum protein levels. There was a significant fat level x sex interaction which was believed to be due to a hormonal difference between the two sexes. The interaction was associated with a reduction in the serum protein level of females as fat was increased, while males showed no consistent effects due to fat. The serum protein levels for females were found to be 5.77, 4.79, and 4.74 g/100 ml and for males were 5.02, 5.18, and 5.00 g/100 ml on the 0, 4, and 8%-fat rations, respectively.

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APPENDIX A

Chromium Sesquioxide Determination

The method followed was that reported by Hill and Anderson (1958).

Reagents

- 1) Concentrated nitric acid
- 2) Concentrated sulfuric acid
- 3) Digestion mixture (prepared as follows: dissolve 10 g sodium molybdate in 150 ml distilled water. Add 150 ml concentrated sulfuric acid slowly. After cooling in an ice bath, add 200 ml 70% perchloric acid with stirring.

Procedure

- 1) Weigh sample, wrap in filter paper, and place in micro-Kjeldahl flask marked at 110 milliliters. When 1.0% Cr_2O_3 is used in the diet, 1 g of diet and 0.5 g of feces are suitable samples.
- 2) Add 10 ml concentrated HNO_3 and allow to stand overnight.
- 3) Heat to dryness on a micro-Kjeldahl digestion apparatus.
- 4) Add 15 ml digestion mixture and heat on micro-Kjeldahl digestion apparatus until color changes from green to yellow, orange, or red. Rotation of the flasks is essential to ensure complete destruction of organic matter and complete oxidation of Cr_2O_3 to chromate.
- 5) Remove flasks, chill in cold water, dilute with 60 ml distilled water, add 20 ml concentrated H_2SO_4 , cool, dilute to volume (110 ml), mix, and allow to stand overnight to permit insolubles to settle.
- 6) Determine the wavelength of maximum absorption by reading optical density of a known Cr_2O_3 mixture at various wavelengths across the scale. (This was done and the absorption curve is shown in Fig. 1.)

- 7) Determine optical density of samples at wavelength of maximum absorption using the Beckman DU Spectrophotometer. Compute Cr_2O_3 content from a reference curve covering the range of 0 to 500 μg of Cr_2O_3 per milliliter. (This was done and is shown as Fig. 2.) The curve has the form $x = 48.25y$ where $x = \text{mg } \text{Cr}_2\text{O}_3 \text{ per 110 ml}$ and $y = \text{optical density}$.

Figure 1. Absorption spectrum for chromium sesquioxide

Figure 2. Standard curve for chromium sesquioxide