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The Nutritive Value of Korean Lespedeza Proteins and the Determination of Biological Values of Proteins for Growing Dairy Heifers

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The increasing importance of Korean lespedeza (Lespedeza stipulacea) as a forage crop in Missouri and other states is well recognized. The increase in popularity of Korean lespedeza, however, has not been accompanied by determinations of digestion coefficients and the efficiency of utilization of its protein. It has been assumed that since Korean lespedeza is a legume similar to alfalfa in crude protein content that it is of equal value to alfalfa and clover in feeding practices (Morrison, 1936). Since legumes are of primary importance in dairy rations because they furnish an abundance of economical protein, one of the first questions regarding a legume forage concerns the nutritive value of its protein. In recent years the acreage of lespedeza in Missouri has become so great that its moderate yield of seed has been viewed as a source of economical protein supplement feed. The need for information concerning the nutritive value of Korean lespedeza hay and seed with special attention to the value of their protein components is thus apparent.

The purpose of the investigations described here, using growing dairy heifers as the experimental subjects, has been to furnish more complete information concerning the nutritive value of the proteins of Korean lespedeza for dairy cattle. The importance of the biological values of feed proteins as a means of expressing their nutritive value for growing cattle has been evaluated.

REVIEW OF THE LITERATURE

Feeding Trials with Korean Lespedeza

Korean lespedeza hay has been compared with other hays in numerous feeding experiments to determine its relative value for growing or milking cattle. McComas, Hazen, and Comfort (1942) found Korean lespedeza hay slightly less valuable than alfalfa or soybean hay for wintering calves. Nevens (1935) reported that a good quality Korean lespedeza hay was equal to alfalfa for wintering dairy heifers.

Korean lespedeza hay in the ration of milking cows has been shown to be inferior to alfalfa hay in experiments reported by Moore and Cowsert (1926), Grinnells (1935), Holdaway and co-workers (1936), and Herman and Ragsdale (1943). Nevens (1935) reported that good quality Korean lespedeza hay was equal in milk producing value to alfalfa hay. Nevens (1934) found that Korean lespedeza straw was definitely inferior to soybean hay for milking cows.

Holdaway and co-workers (1936) estimated digestion coefficients of medium and good Korean lespedeza hays fed to milking cows and calculated that they contained only 39.2 and 43.7 per cent total digestible nutrients respectively.

Herman and Ragsdale (1942) reported that ground Korean lespedeza seed was a satisfactory protein supplement feed for the dairy ration.

Determining the Nutritive Value of Protein

This subject has been very completely reviewed by Mitchell (1924), Mitchell and Hamilton (1929), and Boas Fixsen (1935). Methods used by the early investigators, Osborne and Mendel (1916), Osborne, Mendel and Ferry (1919), and McCollum, Simmonds, and Parsons (1921) were mainly concerned with the gross efficiency of utilization of proteins for growth and other functions. A method was proposed by Thomas (1909) to determine the efficiency of utilization of the nitrogen which was actually absorbed. This method was adapted to experiments with rats by Nevens (1921) and Mitchell and Villegas (1923). Mitchell (1924a), Chick and Roscoe (1930), Boas Fixsen (1930), and Boas Fixsen and Jackson (1932) published similar nitrogen balance methods to be used with rats. Numerous investigators have adapted the rat technique to use with pigs, rabbits, guinea pigs, chickens, mice, sheep and steers. Methods used with the last two species, ruminants, are of special interest in this investigation and will be discussed further below.

The percentage of absorbed nitrogen utilized by the body is generally designated the biological value (a term coined by Thomas, 1909) of the protein. Mitchell (1924b), Mitchell and Villegas (1923), Morgan (1931), Sotola (1930a), Boas Fixsen and Jackson (1932a), Morris and Wright (1935) and Turk and co-workers (1934), among others, have shown that the biological value of any protein is not a constant but varies with the level of protein fed and the relative requirements of the experimental animals. Biological values can express the relative nutritive value of proteins, therefore, only when the proteins are fed to the same or similar animals at the same proportion of the ration. Mitchell, Burroughs, and Beadles (1936) demonstrated that biological values determined with the above facts in mind were accurate and absolutely significant in showing differences in the food value of proteins for rats. Harris and Mitchell (1941a) also confirmed the significance of biological values for sheep.

Mitchell and Villegas (1923) and Mitchell (1924 and 1926) used the biological value, which expresses the percentage of protein not wasted in metabolism, and the coefficient of apparent digestibility, which expresses the percentage not lost from the intestines, to calculate the net protein value of the feed. This method has been used also by Sotola (1930) and Nehring and Schramm (1940). Mitchell (1927) revised the method of calculating the net protein value of the feed to improve its accuracy by using the true digestibility of the protein, upon which the biological value is based, rather than the apparent digestibility. Since most proteins differ more in biological value than they do in digestibility, the biological value has often been used alone to express the nutritive value of a protein. Braman (1931) showed the error of such expression in a comparison of the proteins of linseed and cottonseed for rats. Although they gave the same biological value, linseed protein was more digestible and resulted in better growth.

Determination of Fecal Metabolic Nitrogen

The most difficult and controversial part of the method of determining the biological values of proteins has been the estimation of nitrogen excretion of body origin. These fractions of the excretory nitrogen have been termed *fecal metabolic nitrogen* and *endogenous urinary nitrogen.*

Mitchell (1924a) determined the amount of feces nitrogen per gram of dry feed intake during a nitrogen-free feeding period and used that figure to estimate fecal metabolic nitrogen excreted in subsequent feeding periods. Mitchell and Carman (1926) found that a small amount of whole egg protein added to the nitrogen-free diet stimulated nearly normal food consumption and did not increase the fecal metabolic nitrogen.

Boas Fixsen and Jackson (1932) found that with adult rats and a small feed intake the fecal metabolic nitrogen was not definitely related to feed intake. Schneider (1934) found correlations between fecal metabolic nitrogen and both feed intake and body weight. However, with average sized rats taking a normal amount of feed daily the fecal metabolic nitrogen per gram of feed intake was nearly constant; so it was felt that it was the most logical and only satisfactory method to use in estimating fecal metabolic nitrogen.

Mitchell (1924a), Morgen, Beger and Westhauser (1914) and Hutchinson and Morris (1936) have shown that increasing the fibrous, indigestible portion of the ration results in an increased amount of feces nitrogen per gram of dry feed intake. The following amounts of feces nitrogen per 100 grams of dry feed intake have been reported with ruminants on low-nitrogen rations: Sotola (1930) with sheep, 0.65 grams; Turk, Morrison and Maynard (1934) with sheep, 0.56 gram; Morgen, Beger and Westhauser (1914) with sheep, 0.51 gram; Miller and Morrison (1939) with sheep, 0.55 gram; Harris and Mitchell (1941) with sheep, 0.555 gram; Steenbock, Nelson and Hart (1915) with calves, 0.63 gram; Morris and Wright (1933) with a steer, 0.405 gram; Morris and Wright (1935) with cattle, 0.33 gram; and Hutchinson and Morris (1936) with three cows, 0.48, 0.44 and 0.42 gram respectively. The investigators just referred to have also reported urinary nitrogen data, some of which are probably endogenous, secured from the low-nitrogen rations.

Determination of Endogenous Urinary Nitrogen

The determination of endogenous urinary nitrogen and its use in calculating biological values of proteins is based upon the constancy of a true minimum endogenous catabolism of nitrogen independent of the exogenous nitrogen metabolism. A comprehensive review of this question has been presented by Mitchell and Hamilton (1929).

Mitchell (1924a), Sotola (1930), and Turk, Morrison and Maynard (1934) determined the endogenous nitrogen excretion per unit body weight during a nitrogen-free diet and applied those values to animals in subsequent feeding periods. Smuts (1935) showed, however, that endogenous nitrogen excretion, the same as basal metabolism, bore a more direct relationship to surface area than to body weight. Harris and Mitchell (1941) determined the endogenous nitrogen excretion of their lambs on the basis of surface area. Smuts and Marais (1938 and 1939) and Du Toit and Smuts (1941) calculated that the endogenous nitrogen excretion varied with the 0.734 power of body weight, which value was taken from the basal metabolism and weight relationship reported by Brody, Proctor and Ashworth (1934) for mature animals of different species.

The time of feeding a nitrogen-free diet necessary to reach the endogenous level of nitrogen excretion was shown by Smuts (1935) to depend upon the size of the animal, about five days being required for mice and twenty days for pigs. Du Toit and Smuts (1941) reported that the pig reached the endogenous level by the sixth day of nitrogen-free feeding. Miller and Morrison (1942a) found that lambs required ten to twelve days to reach the endogenous level as compared with six to fifteen days reported by Smuts and Marais (1938). The time necessary to reach the endogenous level was shown by Ashworth and Brody (1933, 1933a and 1935) to depend upon the level of protein in the diet fed previous to the low-nitrogen feeding.

The unpalatability of low-nitrogen diets often results in insufficient feed intake to cover the energy needs with the result that body tissue is catabolized for this purpose. This makes it impossible to determine the true endogenous excretion. Large experimental animals with a store of fat for such emergency use were therefore preferred by Boas Fixsen (1935) and Marais and Smuts (1940). Mitchell and Carman (1926) secured nearly normal feed intake with rats without increasing the endogenous nitrogen when a small amount of egg protein was added to the diet. Miller and Morrison (1942a) found that milk protein in the sheep's low-nitrogen ration stimulated satisfactory feed intake but also increased the nitrogen excretion. Because of feed intake difficulties they preferred to estimate the endogenous nitrogen rather than determine it on each animal.

Harris and Mitchell (1941) and Smuts and Marais (1938 and 1939) have reported low-nitrogen rations which were consumed satisfactorily by sheep. Hart, Humphrey and Morrison (1912) apparently secured satisfactory intake of a ration of straw, sugar, starch, and minerals by calves. Hutchinson and Morris (1936) also fed a low-nitrogen diet to cows for a short time. The dietary difficulties in determining the endogenous nitrogen excretion of ruminants are, therefore, not insurmountable.

Measuring Nutritive Value of Proteins With Cattle

Numerous accounts of feeding experiments to compare proteins are found in the scientific literature but all will not be reviewed here because of their doubtful value in actually expressing differences in *protein* value. Some of the experiments with cattle which were designed so that differences in protein quality would likely appear are reviewed below.

Hart, Humphrey and Morrison (1912 and 1914) compared corn and alfalfa proteins for growing calves and found no difference in utilization. Mitchell (1929) calculated biological values from their data and secured values of 71 for alfalfa and 69 for corn proteins. Hart, Humphrey and Morrison (1914) found that the nitrogen retention of all heifers fluctuated widely from week to week and that it did not correspond to observed body weight gains. The importance of continuing such experiments over a long period was therefore emphasized. They also observed that the utilization of nitrogen was greatly increased during the first week following a low-nitrogen feeding period of four weeks but that it had dropped to normal by the second week.

Morris and Wright (1935) found that the biological values of blood meal, rye, and maize germ meal proteins were similar for growing steers, but the biological value of wheat gluten was slightly inferior to the other proteins.

Hutchinson and Morris (1936a) secured only small differences between the biological values of bean protein and gelatin fed to mature cows recovering from a fast. There was even less difference between the net utilization of the proteins than between the biological values, because the lower biological values were always accompanied by higher digestibility.

Hart and Humphrey (1914, 1914a, 1915, 1916, 1917 and 1918) found only slight differences among the utilizations of the following proteins for milk production: linseed oil meal, distillers grains, casein, milk powder, corn gluten meal, wheat gluten, alfalfa hay, and various mixtures.

Holdaway, Ellet, and Harris (1925) estimated the biological values of peanut meal, cottonseed meal and soybean meal proteins for milk production as 84, 78 and 77 respectively.

Maynard, Miller and Krauss (1928) secured insignificant differences between protein utilization from legume and non-legume rations for milk cows, and they questioned the value of knowing the biological value of protein for dairy rations.

A series of experiments conducted at the Hannah Dairy Research Institute to determine the nutritive value of proteins for milk production were reported by Morris and Wright (1933 and 1933a), Fowler, Morris and Wright (1934), Morris, Wright and Fowler (1936), Morris and Ray (1939) and Morris (1938). In their formula for determining biological value the only actually observed values used were those for milk nitrogen and feces nitrogen. The result was that practically all the variability secured was due to differences in the feces nitrogen. Morris (1938) has plotted the feces nitrogen and biological values showing a direct relation between the two. The conclusion was drawn that the process of digestion, or of excretion into the gut, of nitrogen is dependent upon the animal's needs and the quality of the feed protein. In practically all of the experimental periods the cows showed definite positive nitrogen balances, these being as high or higher on the rations of low "biological value" as on those of high "biological value." The feeds used to furnish the major part of protein for their experimental rations ranked in order of designated quality from highest to lowest were fresh and dried spring grass, grass silage from summer grass, low temperature dried blood meal, fresh and dried autumn grass, bean meal, pea meal, high temperature dried blood meal, meat meal, decorticated earthnut cake, a mixture of the latter and flaked maize, linseed cake, and linseed oil meal.

Mitchell and Hamilton (1929), Boas Fixsen (1935) and Mitchell (1926) have reviewed the difficulties of determining the biological value of protein for any one function, such as milk production, since such functions are never carried on in the animal body to the exclusion of others.

The reported nitrogen balance experiments have shown little real difference in the efficiency of utilization of feed nitrogen by dairy cattle. Feeding trials of longer duration have confirmed this opinion by production results. Hart and Humphrey (1914) secured no difference in milk production between alfalfa nitrogen and corn nitrogen. Bartlett (1936) found no difference between blood meal and wheat gluten for milk production. Salisbury and Morrison (1938) compared a "low quality" protein mixture which was mostly corn and corn gluten meal with "high quality" mixture. No significant difference in production was secured. The use of urea to furnish part of the nitrogen of the ration for milking cows has been reported by Rupel and others (1940) to give production equal to that from rations containing linseed oil meal.

Biological Values of Proteins for Sheep

Sheep have been used widely in determining the quality of proteins for ruminants. Sotola (1930, 1930a and 1933) determined the biological values of alfalfa hay, leaves, and stems, and of sunflower and corn silage. Each was fed alone in rations of varying protein content. Turk, Morrison and Maynard (1934) secured biological values of 50 for alfalfa hay alone and 72 when carbohydrate was added to the ration to adjust it to 10 per cent protein. They also secured (at 10 per cent protein level) biological values of 81 for clover, 79 for alfalfa, 80 for clover and corn, and 77 for alfalfa and corn proteins.

Turk, Morrison and Maynard (1935) secured biological values of 72.8 for soybean oil meal, 65.7 for corn gluten meal, and 67.7 for linseed oil meal at a 10 per cent protein level. Miller and Morrison (1937) compared the same protein supplements fed with timothy hay or corn stover at a 10 per cent level of protein. Values secured with timothy hay were 62 for soybean oil meal, 63 for linseed oil meal, and 64 for corn gluten meal. With corn stover the values were 58 for soybean oil meal, 56 for linseed oil meal and 58 for corn gluten meal.

Miller and Morrison (1939) found that there were very small differences in the utilization of nitrogen from rations containing varying amounts of timothy and alfalfa hays, soybean oil meal and corn. The only significant difference, between alfalfa alone and a mixed ration, was not repeated in a later experiment by Miller and Morrison (1942a). On the basis of extensive experiments with sheep, Miller and Morrison (1942) concluded that no measurable differences in quality of protein were found in rations containing soybean oil meal, corn gluten meal, linseed meal, raw soybeans, unextracted soybean flakes, solvent-process soybean oil meal, "toasted" soybean oil meal, dried skimmilk, casein, or rations in which urea furnished 50 per cent or less of the total nitrogen.

Smuts and Marais (1938a) secured a biological value of 60 for lucerne protein and secured no supplementary effect by the addition of cystine to rations fed to mature wethers. Smuts, Du Toit and v. d. Wath (1941) increased the biological value of lucerne protein from 66 to 76 with young lambs, however, by adding cystine. Smuts and Marais (1939a) secured a biological value of 74 for white fishmeal. Somerset bean protein was found by Smuts, Marais and Bonsma (1940) to have a biological value of 52 when fed alone, 62 when fed with corn and 59 when fed with grass. Smuts and Marais (1940) found that the biological value of grass containing 10 per cent protein was 62 and was increased to 74 for grass containing 7.5 per cent protein.

Harris and Mitchell (1941 and 1941a) found that urea nitrogen was used only slightly less efficiently for growth and maintenance of lambs than casein nitrogen. Johnson, Hamilton, Mitchell and Robinson (1942) compared urea, casein and soybean oil meal supplementation to a low-protein ration for sheep. Biological values of 60.0 and 64.8 were secured for urea, 60.3 for casein and 63.2 for soybean oil meal. On the basis of their observations and reports in the literature they advanced the theory that only small differences in protein utilization are shown by ruminants because a large part of protein utilized by the ruminant is microorganismal protein developed in the paunch from the feed nitrogen regardless of the nature of the latter.

Biological Values for Ruminants and Non-ruminants

Much evidence has accumulated showing that ruminants are less dependent upon the nature of their food nitrogen than are non-ruminants. Mendel (1923) explained that the difference was due to bacterial action which occurred in the rumen. Mitchell and Hamilton (1929) presented convincing data to show that the biological values of feed nitrogen are not different for different species with the exception of ruminants. As data on the biological values of different proteins and mixtures for ruminants have accumulated the difference between ruminants and non-ruminants has become more apparent. Johnson, Hamilton, Mitchell and Robinson (1942) presented the theory that because of the activity of the rumen flora there were very small differences in biological values of different proteins for ruminants. This theory has also received support from Miller and Morrison (1942a). Smuts, Du Toit and v. d. Wath (1941) demonstrated, however, that differences in protein quality can be shown with growing lambs, although such differences are not strictly comparable to results secured with rats.

A comparison of biological values of different proteins for ruminants and non-ruminants shows that ruminants use proteins which are of poor quality for rats with greater efficiency. Thus, while Smuts, Marais, and Bonsma (1940) secured a biological value of 37 with rats for Somerset bean protein, the corresponding value with lambs was 52. On the other hand proteins which are of high quality for rats do not give superior biological values for ruminants. The biological value of milk protein at a 10 per cent level for rats has been calculated at about 85 by Mitchell (1924b); but Miller and Morrison (1942) report that milk was utilized no more efficiently by lambs than other feed proteins which gave biological values of about 62 at a 10 per cent level of crude protein. Furthermore, feeds which demonstrate supplementary action of proteins with rats have not done so with ruminants. Turk, Morrison and Maynard showed that the biological values of alfalfa or clover hay protein alone were the same as when corn was included in the ration. Marais and Smuts (1940a), however, found a marked supplementary action between yellow maize and lucerne for rats.

From their observations made with sheep, pigs and rats, Smuts and Marais (1939a) stated that biological values secured with rats may be applied to pig nutrition but that the application of such data is doubtful with animals like the sheep which have a more complicated digestive tract. Smuts (1938) also wrote, "the value of protein feeds can, therefore, not be estimated or assumed at random from any set of data, but must be determined with different animals for each specific purpose." Boas Fixsen (1935) in a review of the problem of biological values of proteins, concluded that for rats, rabbits, pigs and chickens comparable biological values could be assumed but that such values should not be applied to ruminant nutrition.

In summary, a review of the literature concerning the determination of the nutritive value of protein has revealed the following important information. The nitrogen balance method, or determination of the biological value of the crude protein, has proved to be a satisfactory method of expressing the efficiency of protein utilization with a wide variety of animals including ruminants. This method has shown that the utilization of crude protein by ruminants for growth and maintenance is to a large extent independent of the composition of the protein fed. Nevertheless, some important differences in the utilization of proteins by ruminants have been shown by the biological values. In addition, the biological values have definite biological significance and in conjunction with digestion coefficients they give a valuable measure of the nutritive value of the crude protein of the ration.

MATERIALS AND METHODS

The methods used to determine the nutritive value of Korean lespedeza proteins for dairy heifers were, briefly: (1) determination of the biological value and other measures of protein utilization with the crude protein fed at approximately a 10 per cent level as compared with alfalfa hay protein, (2) determination of supplementary reaction between lespedeza protein and corn, milk and soybean proteins by the comparison of biological values, and (3) determination of the coefficients of digestibility of the nutrients in lespedeza hay and lespedeza seed. Three separate series of experiments were conducted with three groups of four heifers each. Since all of the trials were similar they will be discussed concurrently rather than separately. The term protein as used in this investigation refers to crude protein or 6.25 times the nitrogen content.

Experimental Animals

The animals used were selected from the purebred Holstein-Friesian herd of the Missouri Agricultural Experiment Station. The four animals used in each trial were matched as closely as possible as to age, weight and inheritance. Heifers thirteen to twenty months of age were used because during this age interval the rate of growth is fairly constant. The heifers in the first trial were inseminated during the experiment and one of them later proved to have been pregnant throughout the trial. Since especially discordant data were secured with this heifer, the heifers in the last two experiments were not bred. Data concerning each heifer are given in Table 1. Hereafter each heifer will be identified by her herd number. Photographs taken of the last eight heifers used near the end of the trials are presented in Figs. 1, 2, 3 and 4.

TABLE 1
DATA PERTAINING TO EXPERIMENTAL ANIMALS AT THE START OF THEIR RESPECTIVE EXPERIMENTAL PERIODS

Herd No.	Age	Weight	Gestation	Sire*
	days	Kg.	days	
3	553	383	0	69th
5	546	373	0	Tom
6	547	343	0	Tom
7	546	357	120	Tom
24	506	330	0	69th
26	462	327	0	69th
27	462	320	0	Apostle
28	460	300	0	69th
46	425	323	0	69th
47	398	280	0	69th
48	398	289	0	69th
49	392	303	0	69th

*The sire Apostle was a son of 69th. Tom was practically unrelated to the others.

Body Weight

The heifers were weighed before the evening feeding on each of the three days preceding the collection period and on the day of finishing and the two days following the finish of each collection period. The average weights at the beginning and end of the collection periods were then averaged to determine the average weight during the collection period.

Feedstuffs

The Korean lespedeza hay used in these trials was all grown near Columbia, Missouri, and was relatively free of extraneous material, stubble and weeds having been largely eliminated by clipping the fields while the lespedeza was small. The hay used in the different trials differed in quality as shown by the analyses below.

The alfalfa hay was grown on Missouri River bottomland and was all third cutting and quite free of weeds or other extraneous material.

The wheat straw and oats straw were grown near Columbia, Missouri, and were clean, bright and free of grasses and weeds.

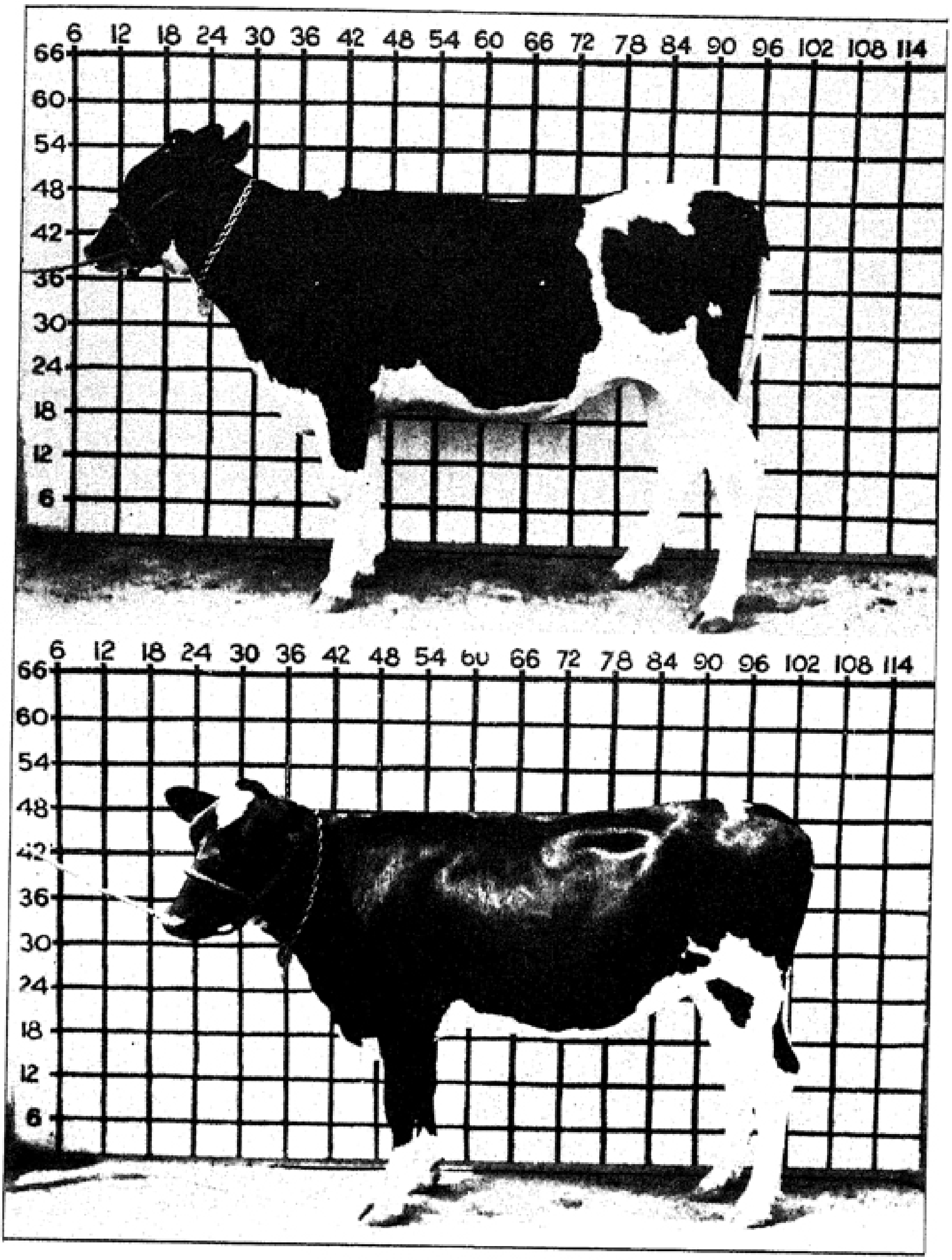


Fig. 1.—Heifer No. 24 (above) and No. 26 (below).

The rest of the feedstuffs used were all secured from the open market. All feedstuffs were secured at one time in supplies large enough to last for at least one series of experiments. Analysis of each feedstuff was made from a composite sample representing all that was used.

Mixing and Feeding Rations

All roughage was chopped moderately fine by passing it through a hammer mill. Concentrates and minerals were thoroughly mixed

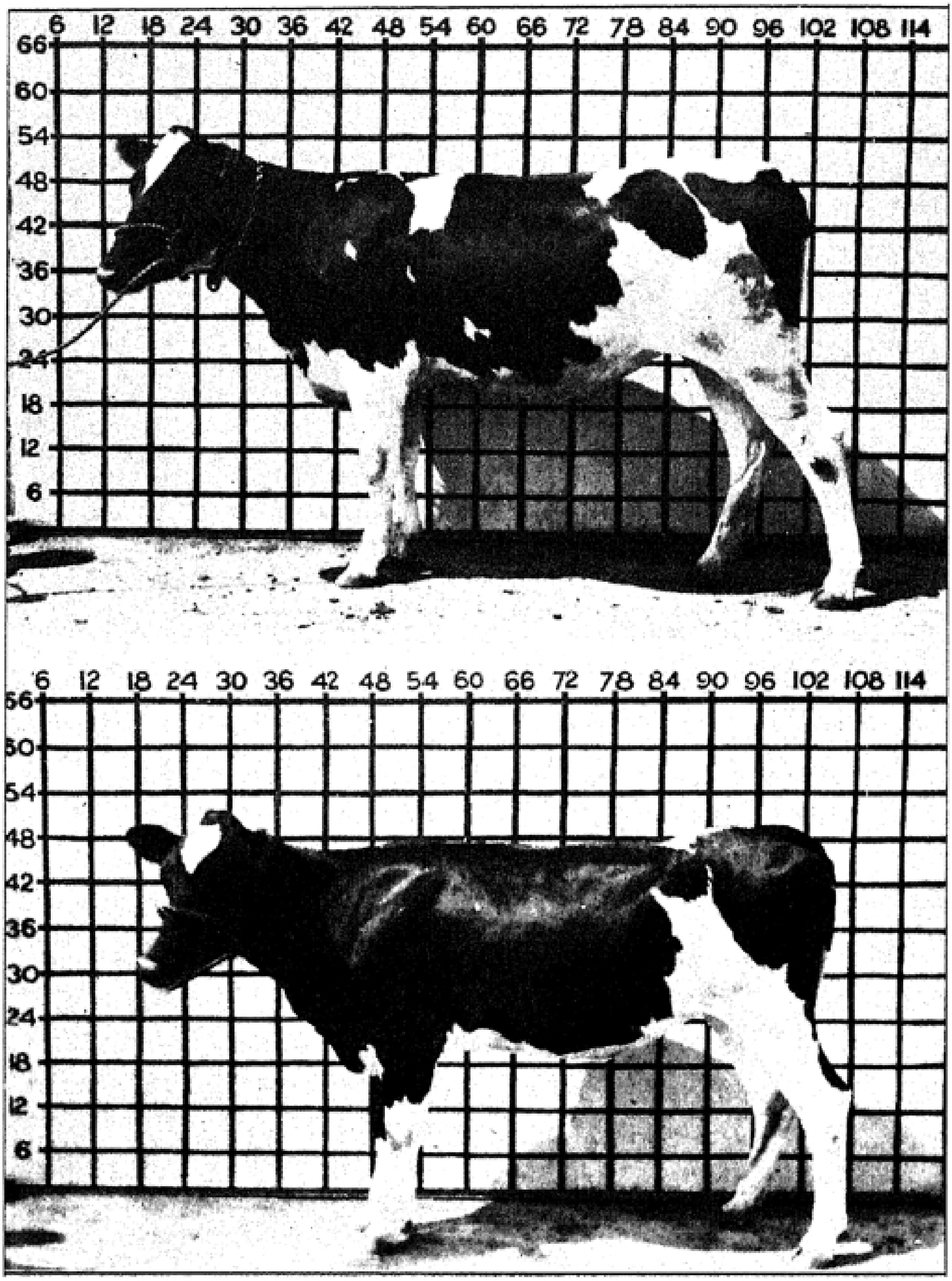


Fig. 2.—Heifer No. 27 (above) and No. 28 (below).

with the chopped roughage if required in the ration and all of any one ration to be used was mixed at the same time. It was then sacked into daily rations for each animal. Moisture determinations were made of the constituents of the ration and the percentage composition of the mixed ration calculated from the composition of its constituents determined from previous analyses.

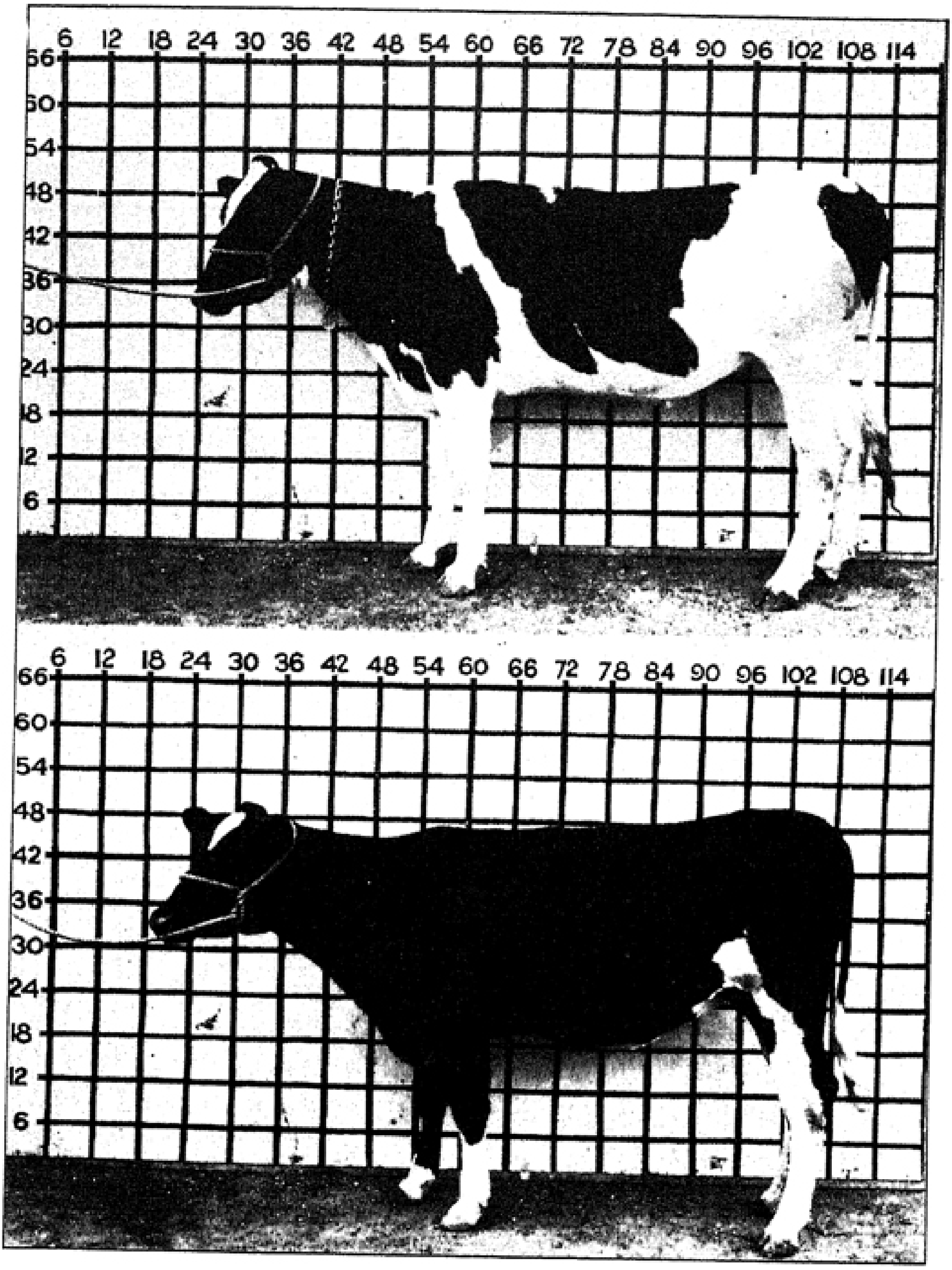


Fig. 3.—Heifer No. 46 (above) and No. 47 (below).

The rations were fed in equal portions twice daily. The animals in the collection stalls were offered water before and after each feeding. During the preliminary feeding period the animals were kept in an experimental feeding barn at night and were turned into a bare lot with free access to water and salt during the day.

If any animal persisted in leaving a significant amount of the ration, the refuse was removed, dried, and weighed, then subtracted

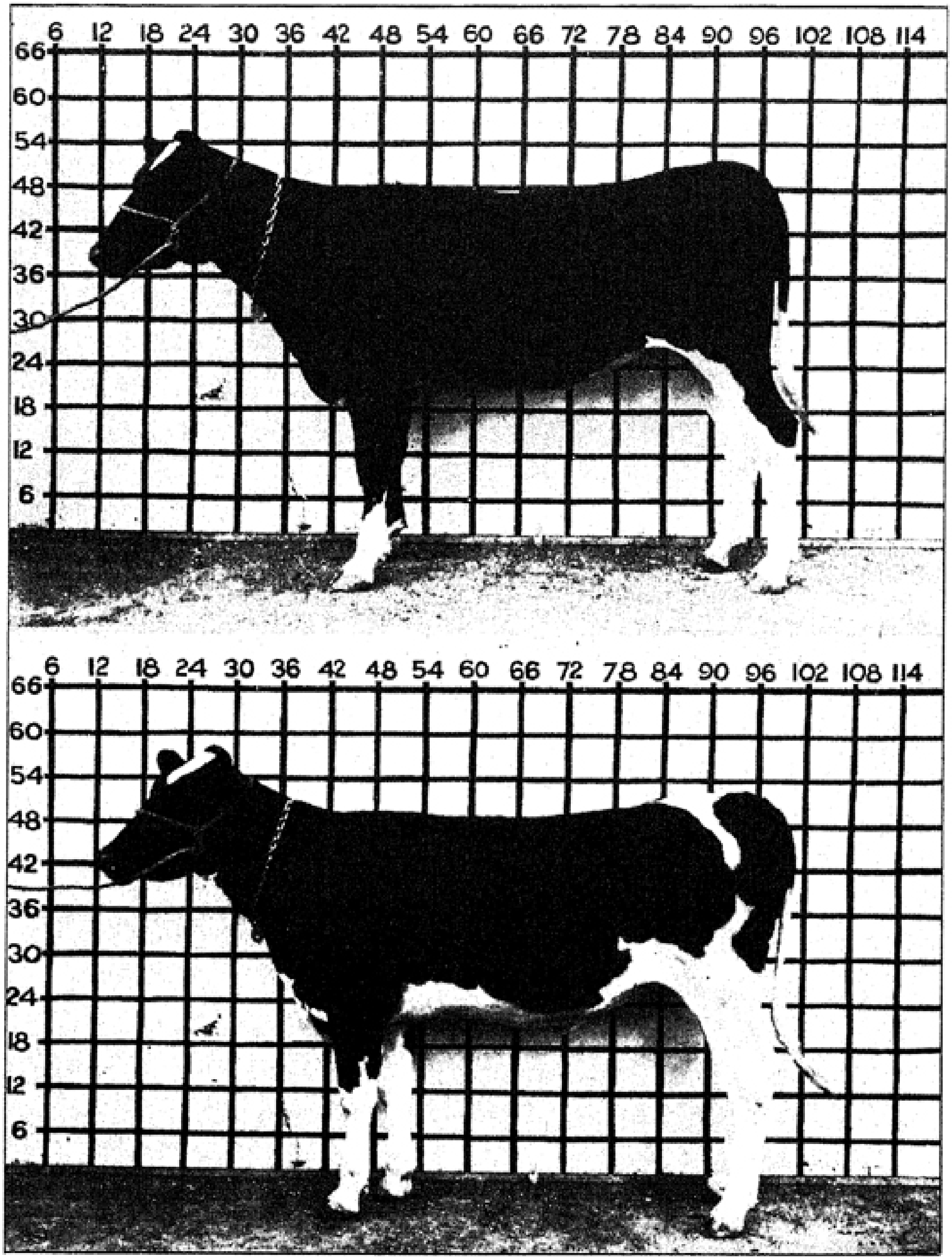


Fig. 4.—Heifer No. 48 (above) and No. 49 (below).

from the total rations to find the amount consumed. The refuse was also analyzed in rations other than the low-protein ration and correction of composition of consumed feed was made if necessary.

Two animals were kept in the collection stalls while the other two were in the preliminary feeding barn. Changes in rations were made abruptly and both preliminary feeding periods and collection periods were of 10 days duration.

The order of feeding the rations was different for each pair during the first trial. Since the order of feeding did not seem to affect the results in this trial, however, the sequence of feeding the rations was kept the same for all four heifers in the subsequent trials.

Collection of Excreta

The collection stalls and the collection apparatus are shown in Figs. 5, 6, and 7. This equipment was very similar to that used and described by Ritzman and Benedict (1929) and Fowler, Morris and Wright (1934). The excreta fell on a rubber belt which was constantly moving slowly on an incline. Feces stayed on the belt and were scraped off into a receptacle. Urine flowed down the belt, was caught in a pan and directed into a large bottle. Since cattle very seldom defecate and urinate simultaneously, separation of feces and urine was practically complete by this method. During the first trial the belts and pans were thoroughly rinsed at the end of the collection period and the rinsings were added to the urine. During the second and third trials the belts and pans were rinsed daily with about two quarts of water, and the rinsings flowed into the urine bottles with the urine.

The floor of the stalls during the first trial was waterproofed canvas over straw and burlap padding. The canvas wore out and had to be replaced often; so during the last two trials a rubber floor matting was used in its place. The floor was sloped so that any urine which happened to fall on the mat flowed into the gutter.

The urine collection bottles were coated with thymol daily and a small amount of dilute hydrochloric acid was added to them during collection periods when the urine nitrogen excretion was very high.

The urine and feces were collected and weighed daily. A three per cent aliquot sample was weighed from each collection and preserved as a composite sample. The composite samples of urine were acidified with dilute hydrochloric acid and were preserved with thymol and refrigeration at 5° C. similar to the method of Hawk and Grindley (1908). The composite samples of feces were kept acidified and moistened with a two per cent solution of hydrochloric acid. They were stored in tight jars and kept in a refrigerator at 5° C. until sampling was completed.

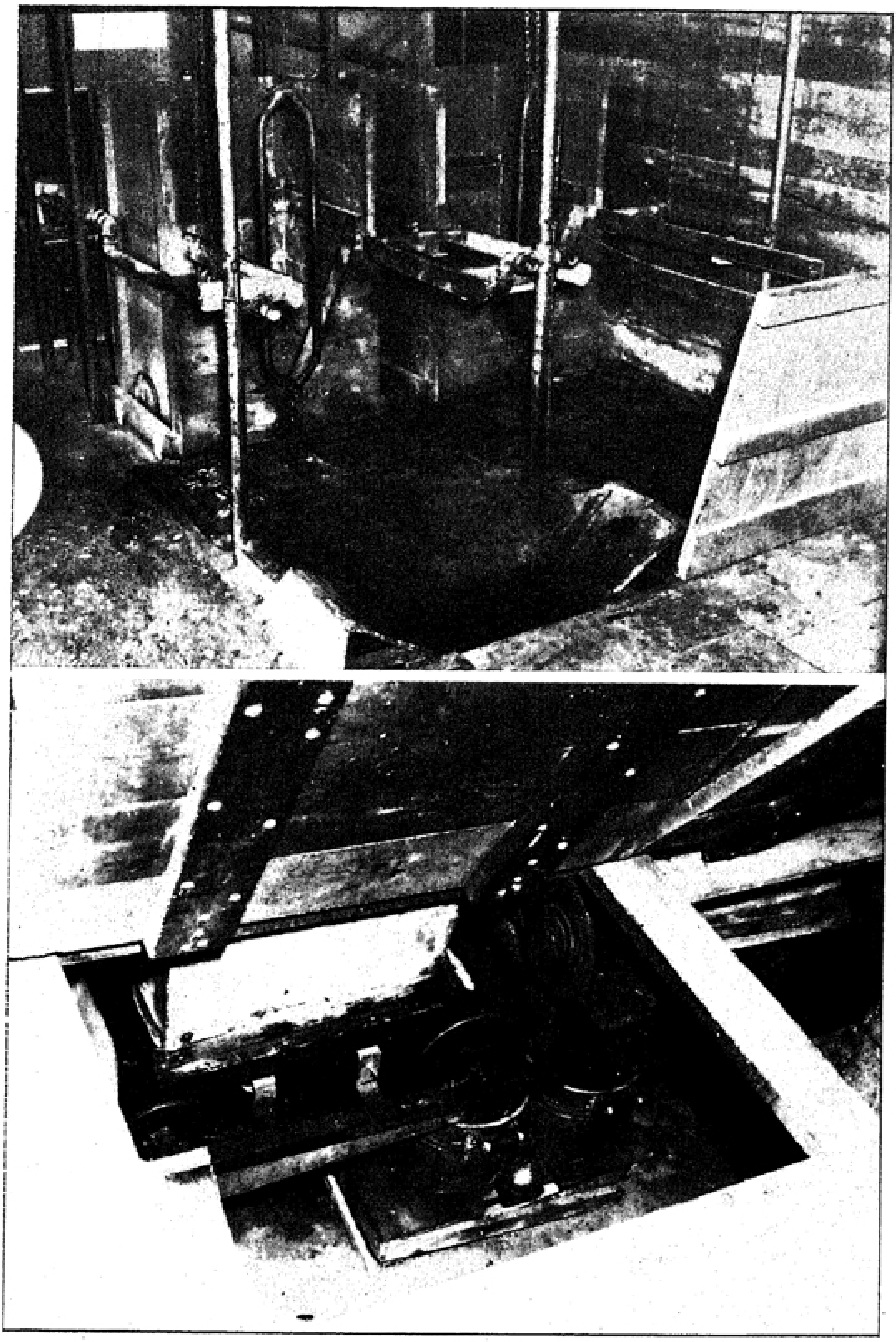


Fig. 5.—Metabolism stalls (above) and collecting apparatus.

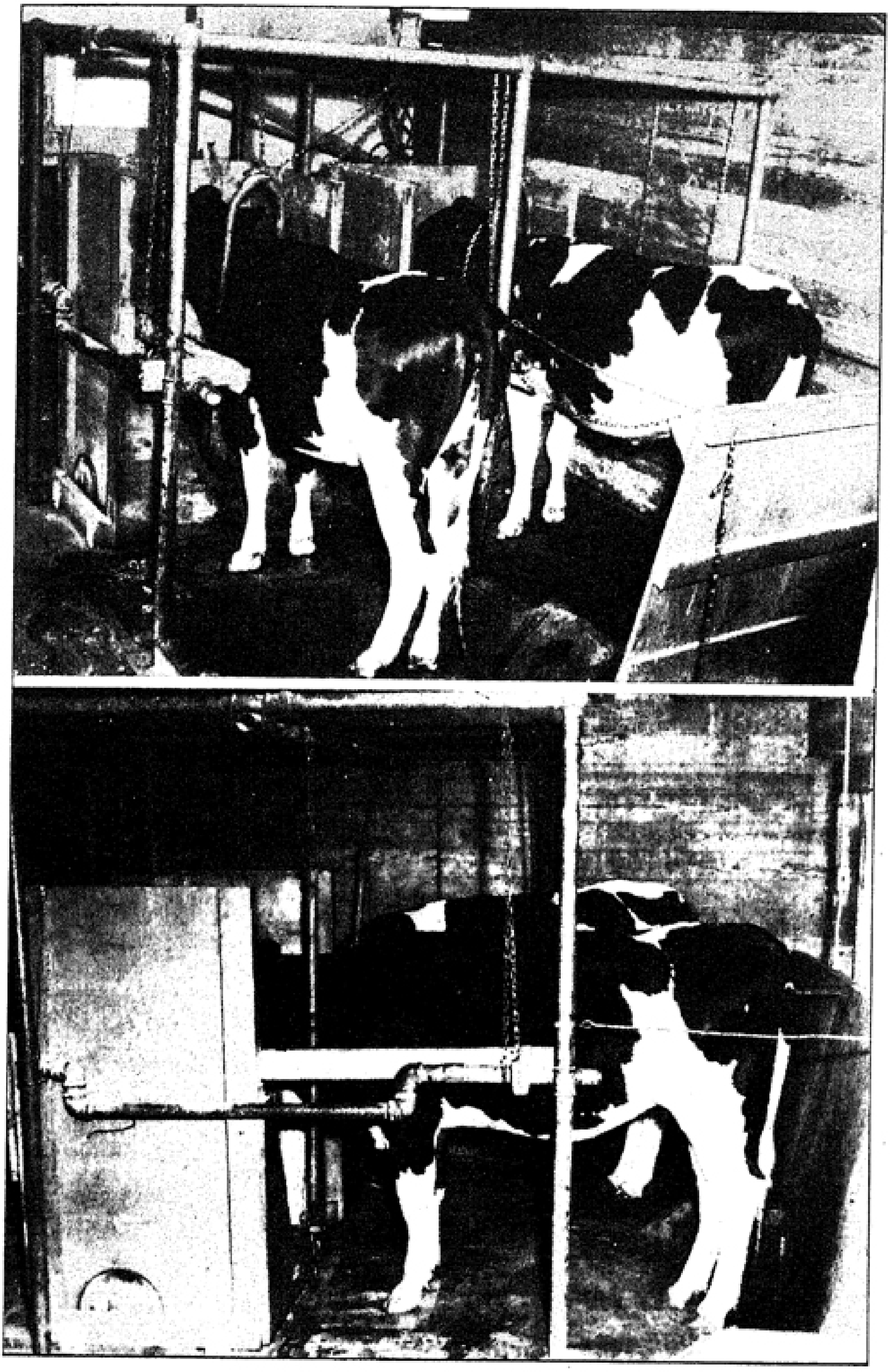
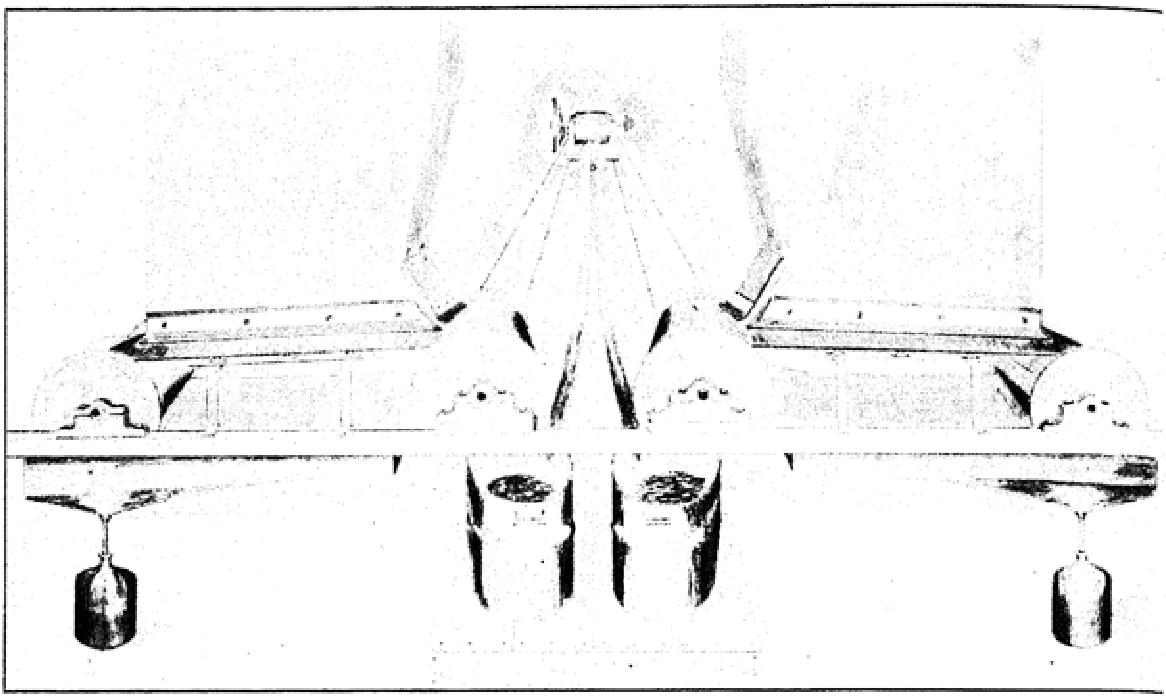


Fig. 6.—Heifers in the collecting stalls.



. Fig. 7.—A sketch of the excreta-collecting apparatus below the metabolism stalls. The motor and gear assembly in the center keep the belts turning continuously toward the center. Feces which fall on the belts are carried through the trapdoors and scraped off into the tubs. Urine flows down the inclined belts and is directed by the funnel-shaped pans into the bottles. This apparatus was patterned after a similar one designed by Ritzman and Benedict (1929). It was originally built and used by Dr. S. Brody, Associate Professor of Dairy Husbanry, University of Missouri, in metabolism investigations.

Methods of Analysis

Urine.—The composite sample of urine was filtered to remove the thymol crystals and then made up to a convenient volume for further analysis. Urine nitrogen was determined by the Kjeldahl-Gunning-Arnold method using 20 or 25 cc samples of urine in triplicate.

Creatinine in urine was determined by a method essentially the same as reported by Peters (1942).

Feeds and Feces.—The composite feces samples were thoroughly mixed and spread out in shallow enameled trays. The feces were then dried in a Freas drying oven at 50° C. with frequent stirring. The dried feces were allowed to reach constant weight in the laboratory air and the amount of air-dry feces determined. The sample was then ground in a Wiley mill, thoroughly mixed, and sampled for analysis.

The feeds and feces were analyzed according to the official methods of the Association of Official Agricultural Chemists (1940) for nitrogen, ash, crude fiber, and ether extract, with nitrogen-free extract determined by difference. Moisture was determined by heating the samples in a drying oven at 105° C. until weighings at two-hour intervals gave no further loss. All nitrogen determinations were in triplicate and the other determinations were in duplicate.

Cellulose was determined by the method of Crampton and Maynard (1938). Lignin was determined by the method of Crampton and Maynard (1938) except that the ether-extracted sample was moistened and autoclaved before it was digested with a 0.5 per cent solution of pepsin in N/10 hydrochloric acid.

Calculating Biological Values of Feed Protein

The biological value was calculated by the formula:

$$\text{Biological value} = \frac{\text{absorbed nitrogen retained by the body} \times 100.}{\text{absorbed nitrogen}}$$

The absorbed nitrogen was determined by subtracting from the total nitrogen intake the feces nitrogen minus the fecal metabolic nitrogen. The absorbed nitrogen retained was determined by subtracting from the absorbed nitrogen the urine nitrogen minus the endogenous urinary nitrogen.

The fecal metabolic nitrogen was calculated on the basis of dry matter intake using the average results secured from all of the low-nitrogen feeding periods. As shown below this was 5.3 grams per kilogram of dry matter consumed.

The endogenous urinary nitrogen was calculated on the basis of the average body weight during the collection period by the formula $N = 0.712\ W^{0.42}$ in which N was grams endogenous urinary nitrogen per day and W was the average body weight in kilograms. This formula was derived from the average relationships between body weight and endogenous urinary nitrogen during periods of low-nitrogen feeding as explained below.

The biological values were also calculated in the first and second trials using values for endogenous urinary nitrogen and fecal metabolic nitrogen which were determined for each heifer from low-nitrogen feeding periods at the beginning and end of the trials. The endogenous urinary nitrogen per kilogram body weight and fecal metabolic nitrogen per kilogram dry matter consumed were assumed to change in a linear fashion from the first to the last low-nitrogen period.

Calculating Other Measures of Protein Nutritive Value

The amount of nitrogen stored was determined from the nitrogen balance. This amount was also expressed as percentage of the nitrogen intake, the utilized nitrogen, and the digested nitrogen.

The stored nitrogen plus the endogenous urinary nitrogen and fecal metabolic nitrogen was termed utilized nitrogen. This was the same as the absorbed nitrogen retained by the body which was previously explained.

The two measures of nitrogen absorption used were the coefficient of apparent digestibility, which was calculated in the usual manner, and the coefficient of true digestibility. The latter was the percentage of nitrogen fed which was absorbed, using 5.3 grams per kilogram dry feed intake for fecal metabolic nitrogen.

The net protein value was determined by the two methods which had been proposed by Mitchell (1926 and 1927). The first method was multiplication of the coefficient of apparent digestibility by the biological value as per cent. This was termed the apparent net protein value. It was used in relation to the nitrogen intake as a comparative measure of the net worth of the crude protein. The true net protein value was determined according to the method outlined by Mitchell (1927) with slight variation. The method of calculation was to subtract the percentage of dry matter in the ration times 3.31 from the product of the percentage of crude protein in the ration times the coefficient of true digestibility times the biological value as per cent. The figure 3.31 represented the fecal metabolic nitrogen per 100 grams dry matter (0.53 gram) times 6.25 to make it crude protein equivalent. The resulting true net protein value was a figure applicable to the amount of feed fed to find the amount of protein or nitrogen utilized outside of losses occurring from the intestines.

Determination of Coefficients of Apparent Digestibility

The handling of rations, length and arrangement of collection periods and handling of excreta were the same for the digestibility studies as for the nitrogen balance experiments. In determining the coefficients of apparent digestibility of hays, the rations were solely chopped hay. One part of ground lespedeza seed was mixed with three parts of hay for calculating the apparent digestibility of lespedeza seed by difference. The coefficients of apparent digestibility were calculated in the usual manner.

EXPERIMENTAL RESULTS

Analyses of Feeds and Composition of Rations

The compositions of the feedstuffs are given in Table 2. These are given on a dry matter basis because the materials differed in percentage of moisture from time to time as the different rations were mixed.

TABLE 2

COMPOSITION OF FEEDSTUFFS

Feedstuffs	Used in Ration No.	Percentage Composition on Dry Matter Basis							
		Crude Protein	Crude Fiber	Nitrogen-free Extract	Ether Extract	Mineral Matter	Lignin	Cellulose	Other Carbohydrates
		%	%	%	%	%	%	%	%
Wheat straw	1,5	3.53	43.26	43.45	1.14	8.61			
Oat straw	6,11,12	3.95	40.48	44.37	1.63	9.57	15.84	40.09	28.92
Oat straw	13,14,15,19	3.83	44.04	42.09	1.83	8.21	17.19	41.70	27.23
Alfalfa hay	2	18.35	26.60	43.45	2.12	9.49	10.72	28.14	31.19
Alfalfa hay	9	16.87	30.31	41.45	2.53	8.85	11.58	35.31	24.86
Lespedeza hay	3,4	17.31	30.56	44.12	2.46	5.56	18.91	30.81	24.95
Lespedeza hay	8	15.53	29.89	46.45	3.17	4.96	21.48	32.89	21.97
Lespedeza hay	7,10	12.45	30.40	49.14	3.70	4.32	16.56	32.76	30.21
Lespedeza hay	20,22	12.42	32.17	46.92	3.16	5.33	15.93	30.96	32.20
Lespedeza hay	16,17	13.08	29.92	49.92	2.53	4.55	17.09	29.00	33.76
Lespedeza hay	18	12.36	33.51	46.23	2.60	5.30	17.33	30.29	32.12
Lespedeza hay	21,23	13.29	37.02	41.49	2.76	5.45	23.14	33.13	22.24
Corn	4,5	11.07	2.66	79.71	4.87	1.69			
Corn gluten meal	5	45.62	6.62	39.87	4.13	3.76			
Corn starch	1 to 6	0.38	0	99.44	0.09	0.09	0	0	99.44
Corn starch	6,8 to 13	0.58	0	99.20	0.11	0.11	0	0	99.20
Corn starch	13 to 20	0.47	0	99.14	0.17	0.11	0	0	99.14
Dextrose	13	0	0	100.00	0	0	0	0	100.00
Sucrose	1 to 17	0	0	100.00	0	0	0	0	100.00
Salt	1 to 20	0	0	0	0	100.00	0	0	0
Steamed bone meal	1 to 6	12.04	2.45	17.21	9.85	68.76			
Steamed bone meal	6, 8 to 13	11.61	2.62	7.93	13.89	63.94			
Steamed bone meal	13,14,15	11.31	2.96	2.77	4.16	78.80			
Soybean oil meal	14,15,18	46.14	6.50	32.12	4.82	10.42	1.32	9.05	28.25
Dried skimmilk	10,11	37.16	0	53.01	1.34	8.49	0	0	53.01
Dried skimmilk	17,19	39.12	0	50.56	1.91	8.41	0	0	50.56
Lespedeza seed	12,20	36.39	10.28	37.58	9.95	5.80	5.67	13.29	28.89
Lespedeza seed	21	39.42	13.88	34.83	5.67	6.21	10.33	15.18	22.98

The compositions of the rations are given in Table 3. Certain peculiarities of these rations will be discussed in connection with the results obtained from feeding them.

TABLE 3

PERCENTAGE COMPOSITION OF RATIONS

Ration 1	%	Ration 2	%	Ration 3	%
Wheat straw	45.000	Alfalfa hay	62.400	Lespedeza hay	62.400
Sucrose	29.000	Sucrose	18.500	Sucrose	18.500
Corn starch	25.000	Corn starch	18.500	Corn starch	18.500
Salt	0.400	Salt	0.300	Salt	0.300
Bone meal	0.600	Bone meal	0.300	Bone meal	0.300
Nitrogen	0.253	Nitrogen	1.647	Nitrogen	1.564
Crude protein	1.580	Crude protein	10.290	Crude protein	9.780
Dry matter	92.600	Dry matter	91.110	Dry matter	91.480
Ration 4		Ration 5		Ration 6	
Lespedeza hay	55.000	Wheat straw	40.000	Oat straw	45.000
Yellow corn	15.000	Yellow corn	20.000	Sucrose	26.000
Sucrose	10.000	Corn gluten meal	15.000	Corn starch	25.000
Corn starch	17.000	Sucrose	15.000	Salt	2.000
Cellu-flour	2.400	Corn starch	9.000	Bone meal	2.000
Salt	0.300	Salt	0.400	Nitrogen	0.305*
Bone meal	0.300	Bone meal	0.600	Dry matter	92.480*
Nitrogen	1.605	Nitrogen	1.505	Nitrogen	0.295**
Crude protein	10.030	Crude protein	9.410	Crude protein	1.840**
Dry matter	90.150	Dry matter	90.500	Dry matter	90.960**
Ration 7		Ration 8		Ration 9	
Lespedeza hay	80.000	Lespedeza hay	71.000	Alfalfa hay	66.000
Sucrose	19.400	Sucrose	13.000	Sucrose	20.000
Salt	0.600	Corn starch	15.000	Corn starch	13.000
		Salt	0.600	Salt	0.600
		Bone meal	0.400	Bone meal	0.400
Nitrogen	1.410	Nitrogen	1.588	Nitrogen	1.577
Crude protein	8.820	Crude protein	9.930	Crude protein	9.860
Dry matter	90.800	Dry matter	90.550	Dry matter	90.400
Ration 10		Ration 11		Ration 12	
Lespedeza hay	59.000	Oat straw	46.000	Oat straw	45.000
Dried skimmilk	9.500	Dried skimmilk	24.300	Lespedeza seed	25.000
Corn starch	20.500	Corn starch	24.000	Corn starch	15.000
Sucrose	10.000	Sucrose	5.000	Sucrose	14.000
Salt	0.600	Salt	0.600	Salt	0.600
Bone meal	0.400	Bone meal	0.100	Bone meal	0.400
Nitrogen	1.603	Nitrogen	1.621	Nitrogen	1.610
Crude protein	10.040	Crude protein	10.130	Crude protein	10.070
Dry matter	91.050	Dry matter	90.440	Dry matter	91.080
Ration 13		Ration 14		Ration 15	
Oat straw	45.000	Oat straw	45.000	Oat straw	45.000
Sucrose	12.500	Soybean oil meal	20.200	Soybean oil meal	16.000
Dextrose	7.500	Corn starch	28.800	Corn starch	34.000
Corn starch	31.000	Sucrose	5.000	Sucrose	4.000
Salt	2.000	Salt	0.600	Salt	0.600
Bone meal	2.000	Bone meal	0.400	Bone meal	0.400
Nitrogen	0.302	Nitrogen	1.617	Nitrogen	1.337
Crude protein	1.890	Crude protein	10.110	Crude protein	8.360
Dry matter	91.440	Dry matter	89.740	Dry matter	88.970
Ration 16		Ration 17		Ration 18	
Lespedeza hay	80.000	Lespedeza hay	55.500	Lespedeza hay	58.000
Corn starch	15.000	Corn starch	35.000	Soybean oil meal	8.000
Sucrose	4.400	Dried skimmilk	8.900	Corn starch	33.400
Salt	0.600	Salt	0.600	Salt	0.600
Nitrogen	1.522	Nitrogen	1.600	Nitrogen	1.565
Crude protein	9.510	Crude protein	10.000	Crude protein	9.780
Dry matter	90.500	Dry matter	89.850	Dry matter	88.280
Ration 19		Ration 20		Ration 21	
Oat straw	45.000	Lespedeza hay	75.000	Lespedeza hay	75.000
Dried skimmilk	22.500	Lespedeza seed	25.000	Lespedeza seed	25.000
Corn starch	31.900				
Salt	0.600				
Nitrogen	1.611	Nitrogen	2.663	Nitrogen	2.856
Crude protein	10.070	Crude protein	16.640	Crude protein	17.850
Dry matter	89.820	Dry matter	89.610	Dry matter	89.150
Ration 22		Ration 23			
Lespedeza hay	100.000	Lespedeza hay	100.000		
Nitrogen	1.784	Nitroten	1.847		
Crude protein	11.150	Crude protein	11.540		
Dry matter	89.800	Dry matter	86.870		

*Mixture fed in first low nitrogen period, second trial.
**Mixture fed in second low nitrogen period, second trial.

Determination of Endogenous Urinary and Fecal Metabolic Nitrogen

Rations and Feeding.—The nitrogen excretion in the feces and urine was determined with each heifer in at least one low-nitrogen feeding period. In the first trial considerable difficulty was encountered in composing a ration that would be consumed regularly in the desired amount. The first ration tried was ration 1 with five per cent molasses added. Since the molasses did not result in improved consumption, ration 1 as listed was fed. It was desired to have the heifers consume the same amount of this ration as of the following experimental rations, but it proved so unpalatable that only about one-half as much was regularly consumed. Since a constant feed intake with a minimum of refuse was necessary to get accurate data for feces nitrogen per kilogram dry feed intake, the amount of feed offered was reduced in subsequent low-nitrogen feeding periods. The first group of heifers satisfactorily consumed 12 pounds of ration 1 daily in their second period.

It was observed that minerals were relished by the heifers fed ration 1. Hence, ration 6 was composed containing more minerals as salt and steamed bone meal. Oat straw was substituted for wheat straw because it was more palatable and contained practically no more nitrogen. Ration 6 was fed at the rate of 10 pounds daily to the heifers in the second trial and it was consumed almost perfectly for the full 20-day feeding periods. Ration 13 which was very similar to ration 6 was fed in the third trial. Only heifers 28 and 48 refused a significant amount of these rations and these refusals occurred after a short collection period during full feed intake had been obtained. The heifers were fed oat straw and a mixed grain ration for a few days prior to beginning the low-nitrogen rations. A few handfuls of the grain mixture were mixed in the first feeding of the low-nitrogen rations; otherwise, the change was abrupt and without a transition period.

Method of Determining Endogenous Nitrogen.—The plan of experimentations in the first two trials was to have a low-nitrogen feeding period at the beginning and end and to calculate the change in excretion on a linear basis for estimating endogenous nitrogen for intervening periods. The average excretions of nitrogen in the urine and feces during each of the low-nitrogen feeding periods for all heifers are given in Table 4. From the average body weight of the heifers during the collection periods the nitrogen excretion per kilogram body weight was determined and this was termed the endogenous urinary nitrogen. The fecal nitrogen per kilogram dry matter consumed was considered as fecal metabolic nitrogen. The values in Table 4 are all from the composite samples except the urine nitrogen of heifers 46, 47, 48 and 49 which were the average of daily collections after a constant low level of excretion had been reached.

TABLE 4

AVERAGE DAILY FEED INTAKE AND FECAL AND URINARY NITROGEN OUTPUT OF HEIFERS ON THE LOW PROTEIN RATIONS

Heifer	Body Weight	Dry Matter Intake	Fecal Nitrogen	Fecal N. per kg. dry matter	Urine Nitrogen	Urine N per kg. Body Weight
No.	Kg.	Kg.	Gm.	Gm.	Gm.	Mg.
3	375.4	3.621	20.313	5.610	9.022	24.03
5	364.7	3.570	22.318	6.252	7.467	20.47
6*	341.6	3.353	20.368	6.075	5.494*	16.08*
7	354.3	3.241	20.117	6.207	9.733	27.47
3	422.5	4.997	23.979	4.818	7.407	17.53
5*	411.4	4.423	25.997	4.423	5.195*	12.63*
6	387.1	4.616	20.852	4.517	8.319	21.49
7	420.9	4.096	22.813	4.650	9.811	23.31
24	328.0	4.195	20.863	4.973	8.387	25.57
26	321.6	4.195	22.631	5.395	8.029	24.97
27	316.6	4.195	21.287	5.074	8.055	25.44
28**	296.0	4.195	19.054	4.542**	9.454	31.94**
24	365.8	4.125	24.742	5.998	8.611	23.54
26	364.9	4.125	22.868	5.544	8.599	23.56
27	353.4	4.125	24.291	5.889	8.347	23.62
28***	328.0	4.125	20.890	5.064	8.577	26.15
46	322.3	4.148	21.379	5.154	8.999	27.92
47	282.8	4.007	23.390	5.837	6.948	24.57
48***	283.3	3.898	19.685	5.050	7.403	26.13
49	305.5	4.148	20.347	4.905	7.116	23.29
Mean				5.300		
Standard Error				0.130		

* Urine Output was very low and collection was unsatisfactory.
** Collection period was only four days. The heifer refused feed thereafter.
*** Collection period was six days that feed intake was satisfactory.

Fecal Metabolic Nitrogen.—The mean excretion of nitrogen in the feces was 5.30 ± 0.130 grams per kilogram of dry matter consumed. The data from heifers 3, 5, 6 and 7 indicate that the fecal nitrogen per kilogram dry matter consumed may decrease as the feed intake increases. During their second low-nitrogen period they consumed nearly 50 per cent more feed than during their first period. Their total feces nitrogen did not increase proportionally, however, which resulted in a decline in the fecal nitrogen per kilogram feed intake. This result may be the effect of a sizeable portion of feces nitrogen not related to the amount of feed but, possibly, to body size or some other factor. The data from heifers 24, 26, 27 and 28 indicate that an increase in body size may result in increased fecal nitrogen on the same feed intake. Their excretion of nitrogen in the feces was greater on practically the same amount of the same ration during their last low-nitrogen period than it was during their first such period when they were four months younger and about 40 kilograms lighter.

Since the data secured here were not sufficient to afford a reasonably accurate estimate of the proportions of fecal nitrogen due to feed or other factors, estimations of the metabolic nitrogen of the feces were made solely on the basis of dry matter intake. It is believed that any error in this method would not be great enough to affect adversely comparisons between rations fed to the same animals.

Urine Nitrogen and Creatinine Excretions.—The daily excretions of total urinary nitrogen and of preformed creatinine were determined for each of the eight heifers used in the last two trials while they were being fed the low-nitrogen ration. The total and creatinine nitrogen data are plotted in Fig. 8 with reference to the length of time the heifers had been consuming only the low-nitrogen ration.

It was noted that there were only minor variations in the excretion of creatinine from day to day with any one heifer. The total nitrogen excretion was more variable than the creatinine nitrogen excretion, but it became fairly constant after the heifers had been fed the low-nitrogen ration for 10 or 11 days. At this time the creatinine nitrogen was about 29 to 30 per cent of the total urine nitrogen. By the use of this ratio it was easy to determine whether or not the heifer had yet reached the endogenous level of nitrogen excretion and also to determine when the nitrogen excretion was unduly increased due to excess catabolism above the endogenous. These conditions could usually be determined also by simply comparing the total nitrogen excretions, but the ratio between total nitrogen and creatinine nitrogen was considered satisfactory confirmatory evidence.

The low level of nitrogen in the preceding rations, which were not over 10 per cent protein, probably contributed to a rapid adjustment of the urinary nitrogen excretion to the endogenous level. From Fig. 8 it can be seen that these heifers had practically reached a constant low level of nitrogen excretion by the tenth day of feeding only the low-nitrogen ration. Heifers 26 and 46 were the only exceptions. There also seemed to be a very slight tendency for the total nitrogen excretion to continue slightly downward during the collection period. A *minimum* urinary nitrogen excretion could probably be secured after a longer period of low-nitrogen feeding than 20 days.

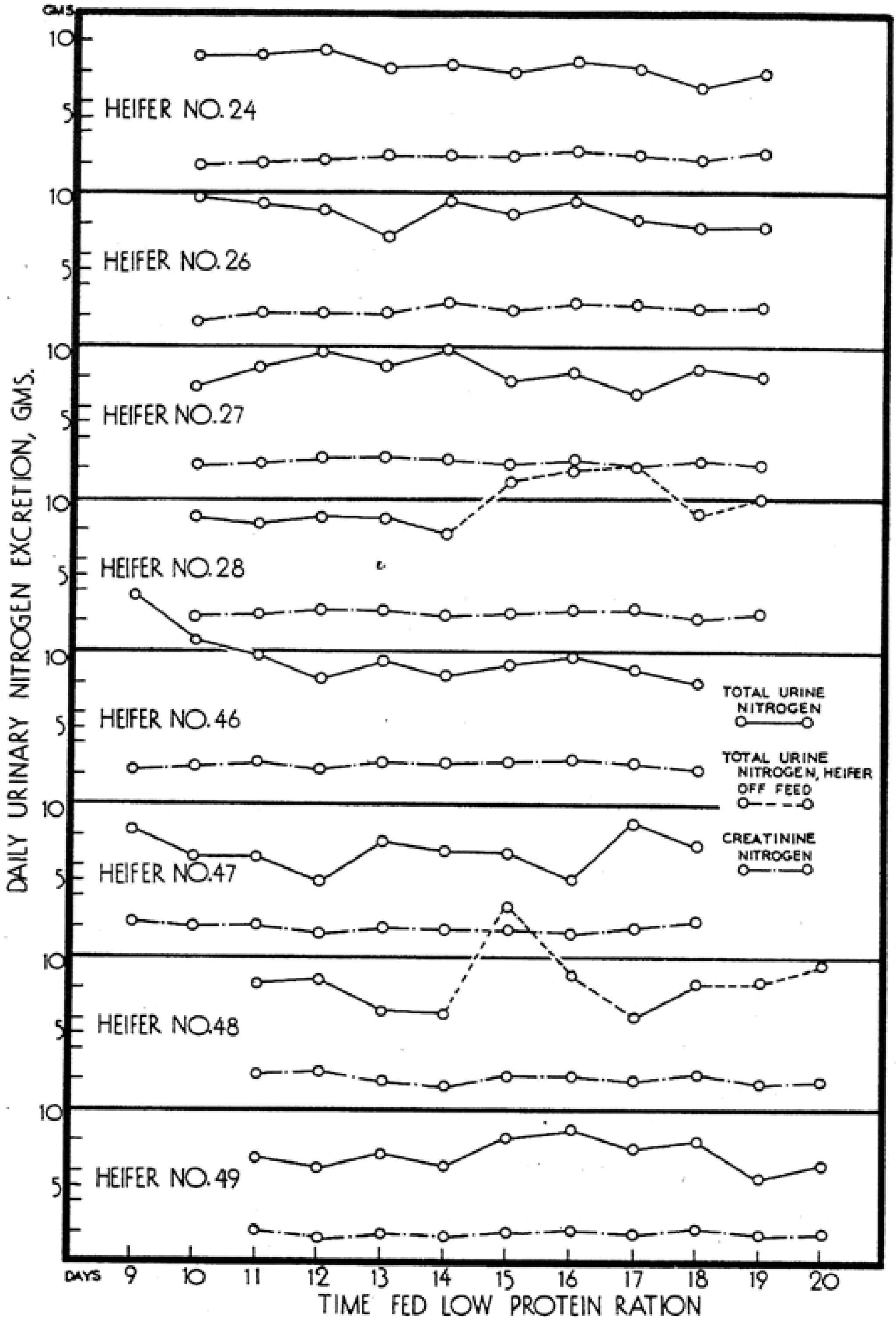


Fig. 8.—Daily excretions of total urine nitrogen and of creatinine nitrogen of eight heifers for their ten-day collection periods plotted against time they were fed only the low-nitrogen ration.

The Effect of Feed Intake upon Urinary Nitrogen.—When heifer 28 began to refuse part of the low-nitrogen ration her total urine nitrogen increased markedly as shown by the broken line in Fig. 8. She continued to refuse feed and did not return to the previous low level of nitrogen excretion. Heifer 48 refused more than 50 per cent of her ration during the fifth collection day and her urine nitrogen excretion more than doubled. On the sixth day she ate the full ration but her urine nitrogen was still high. She continued eating all of her feed until the ninth day and her excretion of nitrogen in the urine fell again to the previous low level then rose as feed was refused again. These instances showed that the consumption of practically the full amount of the low nitrogen ration fed was necessary to prevent excess catabolism of body tissue for energy or carbohydrate production.

It was not determined by experiment whether or not the low-nitrogen rations fed were sufficient to cover the animals' energy needs so that no body tissue was being used for this purpose. The data for heifers 28 and 48, however, indicate that an intake of much less than 10 pounds daily was certainly not enough. Brody, Kibler and Ragsdale (1942) calculated from resting maintenance metabolism determinations that Holstein heifers weighing from 300 to 400 kilograms required 4.5 to 5.1 pounds of total digestible nutrients daily. Since the animals in this experiment were stabled practically all of the time, their maintenance energy requirements should not have been greatly in excess of the resting energy maintenance costs as predicted.

Coefficients of apparent digestibility were determined from the two low-nitrogen feeding periods with heifers 24, 26, 27 and 28. These are presented in Table 5. The calculated digestible nutrients which the low-protein ration furnished are presented in Table 6.

TABLE 5
DIGESTION COEFFICIENTS OF THE LOW PROTEIN RATION

Period	Heifer No.	Dry Matter %	Crude Protein %	Crude Fiber %	Ether Extract %	N-free Extract %
1	24	57.64	-51.15	27.08	23.98	73.65
	26	55.08	-63.98	19.78	12.29	71.62
	27	57.89	-54.26	22.42	29.08	75.10
	28	61.17	-38.32	27.51	50.98	77.84
2	24	50.02	-84.94	4.33	40.02	70.50
	26	54.71	-70.93	12.94	33.93	73.49
	27	51.50	-81.67	9.75	12.38	72.71
	28	54.46	-56.13	11.23	28.73	73.49
Average		55.31	-62.67	16.88	28.92	73.56

TABLE 6

DIGESTIBLE NUTRIENTS IN THE LOW PROTEIN RATION

Period	Heifer	Crude Protein	Crude Fiber	Ether Extract	N-free Extract	TDN
	No.	%	%	%(x2.25)	%	%
1	24	-0.97	4.52	0.62	48.62	52.79
	26	-1.22	3.30	0.32	47.28	49.68
	27	-1.03	3.74	0.75	49.58	53.04
	28	-0.73	4.59	1.32	51.39	56.57
2	24	-1.56	0.69	0.84	46.33	46.30
	26	-1.31	2.08	0.71	48.30	49.78
	27	-1.50	1.57	0.23	47.79	48.09
	28	-1.03	1.80	0.60	48.30	49.67
Average		-1.17	2.79	0.67	48.45	50.74

When the net loss of crude protein in the feces was deducted from the sum of the digestible crude fiber, digestible nitrogen-free extract and 2.25 times the digestible ether extract, an average of 50.74 per cent total digestible nutrients was secured from the eight determinations. This would have been an average total digestible nutrient supply of 5.07 pounds from the daily low-nitrogen ration. The range for the heifers was 4.63 to 5.66 pounds daily. From these calculations it is believed that the low-nitrogen ration as fed probably furnished sufficient energy to obviate any necessity for the heifers to draw upon their body tissues for this purpose. The energy supplied in the total ration was undoubtedly close to the minimum, however as evidence by the comparison with Brody's prediction table and by the immediate increase in urine nitrogen of heifers which refused the ration.

Endogenous Urinary Nitrogen as a Function of Body Weight.—It was observed in the first and second trial that the urinary nitrogen excretion per kilogram body weight was usually less for the same heifer in the second period of low-nitrogen feeding than it was in the first period. This was to be expected since it had been shown with other species that the urinary endogenous nitrogen excretion does not increase 100 per cent for each corresponding 100 per cent increase in body weight. In order to determine the relationship between increasing body weight and endogenous nitrogen for these heifers, the daily nitrogen excretion (Table 4) was plotted against body weight on a logarithmic coordinate chart. The abnormal values secured from heifers 5, 6 and 28 were not used because of unsatisfactory collection periods as explained at the bottom of Table 4. The regression line was fitted to the plotted data by the method of least squares and the index of correlation and standard errors of estimate were computed. These are all given in Fig. 9 as Line II and accompanying data. The data secured in each trial are represented by a different symbol. The data from the first group account for most of the variation. Perhaps the experience gained with this group afforded improvements in technique which should make the values secured with the second and third group of slightly more importance. These latter values are much closer to the average line of regression.

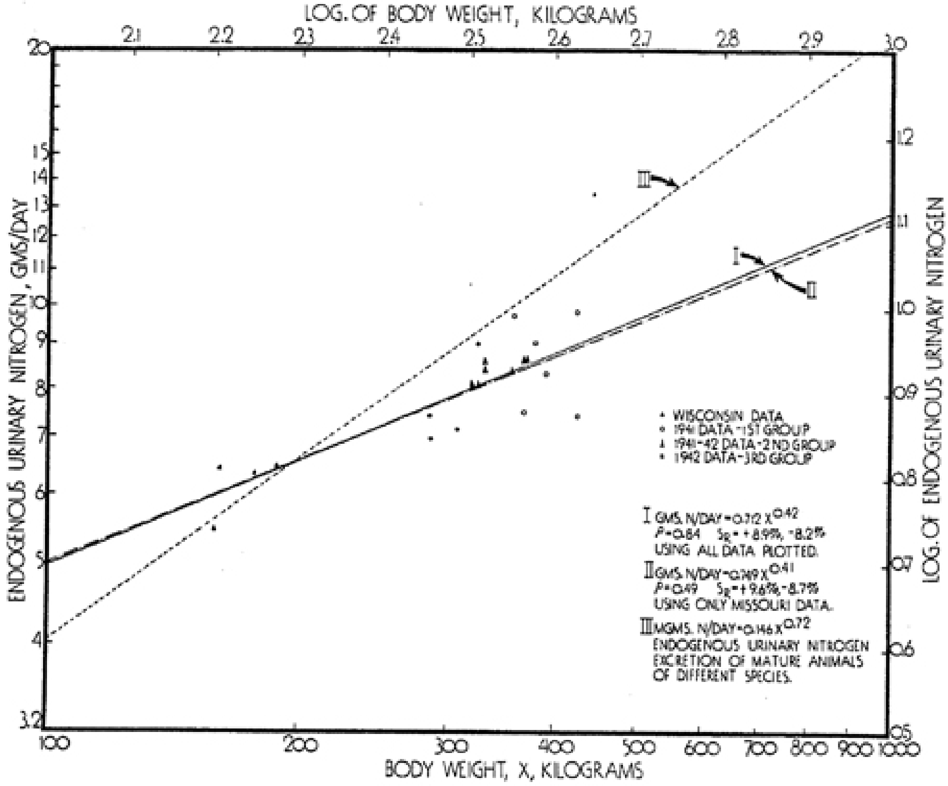


Fig. 9.—Daily excretion of endogenous urinary nitrogen of Holstein heifers plotted against body weight, including the data from this investigation and from the literature. Lines I and II were fitted to the data as stated on the chart, representing the equation $Y = aX^b$. Line III represents endogenous nitrogen excretion for *mature* animals of *different* species as calculated by Brody, Proctor, and Ashworth (1934).

In order to test the accuracy of the line secured, the data published by Steenbock, Nelson and Hart (1915) and Hart, Humphrey and Morrison (1914), which were the only accurate data on the endogenous urinary nitrogen excretion of cattle found in the literature, were plotted on the same chart. Their values fell remarkably close to the line calculated from data secured in these experiments. The average regression line computed from all of the data plotted was drawn as line I. It falls practically on top of line II and results in a higher index of correlation ($\rho = 0.84$) and smaller standard errors of estimate than those for line II. Because of this fact and the greater range in body weight included in fitting line I, the regression equation, $N = 0.712\ X^{0.42}$ for this line was used to compute the endogenous nitrogen for all of the biological value determinations. N is grams of urinary endogenous nitrogen excreted daily and X is body weight in kilograms. The use of this average line recognizes that endogenous nitrogen metabolism does not increase as the first power of body weight. It does not allow for the individual differences of different heifers; yet, on the other hand, it does not distort values secured with heifers because of unsatisfactory performance or unusual results during the low-nitrogen feeding period. In this manner the use of the average equation may gain as much or more accuracy as it loses in calculating average biological values.

The accuracy of the equation used is not beyond question because it is based on relatively few determinations of endogenous urinary nitrogen with animals of a relatively small range in body weight. More determinations with animals weighing between 200 and 300 kilograms and above 400 kilograms would be desirable. It is believed, however, that the exponent of body weight in the equation should be nearer 0.42 than the 0.72 of line III which is from the equation for mature animals of different species computed by Brody, Proctor and Ashworth (1934). All of the points for the heavier animals used in this study fell well below line III. If the endogenous nitrogen of growing animals increases at the same rate as does energy metabolism the exponent may be between 0.50 and 0.60, since Brody, Kibler and Ragsdale (1941 and 1942) have shown that resting metabolism of growing heifers increases as the 0.56 power of body weight for Jerseys and as the 0.60 power for Holsteins after six months of age. It is possible, however, that endogenous nitrogen metabolism does not increase at as high a rate as does resting energy metabolism. If such is the case the equation as secured in this study may be nearly correct.

Biological Value Determinations

Biological values were determined for all rations according to the methods previously given. The various rations described in Table 3 were fed to the heifers in the order shown in Table 7. Data from which the biological values were calculated for each heifer on each ration are presented in Table 8. The rations were listed in the order of their use as nearly as possible except those rations primarily used for the determination of coefficients of digestibility. Data for these were listed at the end of the table. These data have been presented in detail because of the small number of animals involved in each determination.

The biological values in Table 8 were secured by use of the average formulae for estimating endogenous urinary nitrogen and fecal

TABLE 7

ORDER OF FEEDING THE EXPERIMENTAL RATIONS

	Heifers No. 3 and 5		Heifers No. 6 and 7		Heifers No. 24, 26, 27 and 28		Heifers No. 46, 47, 48 and 49	
	No.	Description	No.	Description	No.	Description	No.	Description
Rations in order of feeding	1	Low protein	1	Low protein	6	Low protein	13	Low protein
	2	Alfalfa	3	Lespedeza	7	Lespedeza	16	Lespedeza
	3	Lespedeza	2	Alfalfa	8	Lespedeza	14	Soybean oil meal
	4	Corn-lespedeza	3	Lespedeza	9	Alfalfa	17	Milk-lespedeza
	5	Corn	4	Corn-lespedeza	10	Milk-lespedeza	19	Milk
	1	Low protein	1	Low protein	11	Milk	18	Soybean-lespedeza
					6	Low protein	15	Soybean oil meal
					12	Lespedeza seed	23	Lespedeza hay
					22	Lespedeza hay	21	Lespedeza hay and seed
					20	Lespedeza hay and seed		

metabolic nitrogen, which were discussed in the preceding sections. Since data were also available for estimating these values on the assumption of linear change from the first to the last low-nitrogen feeding period for eight heifers, these were presented in Table 9. The values secured from these data were termed "individual" because they were calculated for each heifer from data secured from that heifer only during her low-nitrogen feeding periods. As can be seen in Table 4, there was considerable variation in the changes which occurred from the first to the second low-nitrogen feeding period. From the average relationships between fecal metabolic nitrogen and dry matter consumption and between endogenous urinary nitrogen and body weight which were disclosed in the preceding sections, these respective values can be accurately estimated by use of the formulae presented. Since these formulae express the average relationships, the values secured by their use have been termed "average" values. These individual data are presented in comparison with similar average values secured by use of the formulae. In most cases, as shown in Table 9 there was close agreement between biological values secured by the two methods. Furthermore, the standard errors of the means were not significantly greater for one method than for the other. In some cases use of the formulae gave less variation and in other cases it gave slightly more variation than did the individual data. In the light of these results the second low-nitrogen feeding period was omitted from the plan for heifers 46, 47, 48 and 49 in order to feed more rations and to expedite the summarization of the data. The formula method was used as a basis of determinations for this lot of heifers and the other heifers as well. A combination of the material presented in Tables 8 and 9 contains the figures necessary for calculating the biological values.

TABLE 8

AVERAGE BODY WEIGHTS, DAILY FEED AND NITROGEN INTAKE, DAILY FECAL AND URINARY NITROGEN EXCRETION, AND BIOLOGICAL VALUES FOR INDIVIDUAL HEIFERS

Ration	Heifer No.	Average Weight	Intake		Feces Nitrogen	Absorbed Nitrogen	Urine Nitrogen	Retained Nitrogen	Biological Value
			Dry Matter	Nitrogen					
		kg.	kg.	gm.	gm.	gm.	gm.	gm.	%
2 Alfalfa hay	3	383.1	6.199	112.060	55.795	89.120	32.519	65.258	73.23
	5	379.0	6.199	112.060	49.199	95.716	25.263	79.072	82.61
	6	361.3	5.992	108.325	46.349	93.761	27.371	74.834	79.81
	7*	385.6	6.199	112.060	45.330	99.585	25.785	82.485	82.83
	Average	374.5	6.130	110.815	50.447	92.866	28.384	73.055	78.55
3 Lespedeza hay	3	396.2	6.224	106.413	57.676	81.724	19.324	71.179	87.10
	5	392.6	6.224	106.413	54.194	85.206	17.188	76.769	90.10
	6_1*	349.5	6.017	102.866	60.120	74.636			
	6_2	373.5	6.017	102.866	58.968	75.788	16.690	67.659	89.27
	7_1*	365.1	6.017	102.866	58.333	76.423	9.984	74.923	98.04
	7_2*	403.5	6.224	106.413	60.275	79.125	14.335	73.634	93.06
	Average	387.4	6.155	105.231	56.946	80.906	17.734	71.869	88.82
4 Lespedeza hay and corn	3	404.2	6.134	109.203	59.583	82.129	22.128	68.854	83.84
	5	394.2	6.134	109.203	63.926	77.786	19.543	67.004	86.14
	6	382.8	5.929	105.563	61.821	75.166	16.648	67.175	89.37
	7*	423.4	6.134	109.203	61.665	80.047	13.798	75.275	94.04
	Average	393.7	6.066	107.990	61.777	78.360	19.440	67.678	86.45
5 Corn	3	419.1	6.158	102.399	45.720	89.314	35.808	62.496	69.97
	5	411.0	6.158	102.399	47.448	87.586	22.414	74.089	84.59
	Average	415.1	6.158	102.399	46.584	88.450	29.111	68.293	77.28
7 Lespedeza hay	24	329.1	6.460	100.431	63.364	71.305	17.864	61.563	86.34
	26	329.8	6.192	95.886	59.871	68.830	15.509	61.453	89.28
	27	318.2	6.590	102.331	62.639	74.618	17.196	65.428	87.68
	28	303.2	6.590	102.331	57.570	79.687	16.259	71.274	89.44
	Average	320.1	6.458	100.245	60.861	73.610	16.707	64.929	88.19
8 Lespedeza hay	24	327.7	6.161	108.062	63.025	77.690	22.098	63.703	82.00
	26	331.8	6.161	108.062	64.387	76.328	16.204	68.277	89.45
	27	322.1	6.161	108.062	62.951	77.764	22.206	63.607	81.80
	28	301.2	6.161	108.062	52.702	88.013	22.160	73.666	83.70
	Average	320.7	6.161	108.062	60.766	79.948	20.667	67.313	84.24
9 Alfalfa hay	24	353.6	6.151	107.299	49.895	90.003	33.644	64.734	71.92
	26	351.1	6.151	107.299	56.436	83.462	33.161	58.646	70.27
	27	333.6	6.151	107.299	55.689	84.209	28.971	63.411	75.30
	28	316.6	6.151	107.299	51.292	88.606	30.945	65.657	74.10
	Average	338.7	6.151	107.299	53.330	86.570	31.680	63.112	72.90
10 Lespedeza hay and milk	24	357.7	6.195	109.069	57.048	84.855	24.009	69.261	81.62
	26	359.0	6.195	109.069	55.356	86.547	24.181	70.791	81.80
	27	344.3	6.195	109.069	58.157	83.746	20.315	71.706	85.62
	28	324.3	6.195	109.069	55.986	85.917	20.994	72.993	84.96
	Average	346.3	6.195	109.069	56.637	85.266	22.375	71.188	83.50
11 Milk	24	367.6	6.154	110.292	48.242	94.664	40.298	62.879	66.42
	26	372.2	6.154	110.292	43.163	99.743	42.944	65.351	65.52
	27	361.3	6.154	110.292	48.054	94.852	34.451	68.845	72.58
	28	340.9	6.154	110.292	53.669	89.237	32.521	64.961	72.80
	Average	360.5	6.154	110.292	48.282	94.624	37.554	65.509	69.33
12 Lespedeza seed	24	366.3	6.197	109.544	54.362	88.026	33.919	62.600	71.12
	26	378.3	6.197	109.544	51.163	91.225	37.197	62.637	68.66
	27	363.3	6.197	109.544	55.429	86.959	30.530	64.893	74.63
	28	341.8	6.197	109.544	50.873	91.515	28.554	71.206	77.81
	Average	362.4	6.197	109.544	52.957	89.431	32.550	65.334	73.06

*Records of these periods were not included in the average of the respective rations.

TABLE 8 (continued)

AVERAGE BODY WEIGHTS, DAILY FEED AND NITROGEN INTAKE, DAILY FECAL AND URINARY NITROGEN EXCRETION, AND BIOLOGICAL VALUES FOR INDIVIDUAL HEIFERS

Ration	Heifer No.	Average Weight	Intake		Feces Nitrogen	Absorbed Nitrogen	Urine Nitrogen	Retained Nitrogen	Biological Value
			Dry Matter	Nitrogen					
		kg.	kg.	gm.	gm.	gm.	gm.	gm.	%
16 Lespedeza hay	46	325.9	6.158	103.555	58.505	77.685	22.423	63.352	81.55
	47	299.4	6.158	103.555	67.229	68.961	13.508	63.255	91.73
	48	289.4	6.158	103.555	61.084	75.106	18.491	64.306	85.62
	49	301.4	6.158	103.555	57.116	79.074	23.351	63.547	80.36
	Average	304.0	6.158	103.555	60.984	75.207	19.443	63.615	84.82
14 Soybean oil meal	46	339.3	6.106	110.019	44.007	98.373	29.721	76.876	78.15
	47	313.0	6.106	110.019	46.629	95.751	28.438	75.266	78.61
	48	304.1	6.106	110.019	50.092	92.288	24.860	75.285	81.58
	49	319.1	6.106	110.019	49.191	93.189	29.975	71.231	76.44
	Average	318.9	6.106	110.019	47.480	94.900	28.249	74.665	78.70
17 Lespedeza hay and milk	46	355.4	6.113	108.862	55.694	85.569	25.734	68.220	79.73
	47	324.8	6.113	108.862	59.566	81.697	21.054	68.723	84.12
	48	320.7	6.113	108.862	58.890	82.373	22.535	67.876	82.40
	49	332.7	6.113	108.862	56.461	84.802	26.126	66.839	78.82
	Average	333.4	6.113	108.867	57.653	83.610	23.862	67.915	81.27
19 Milk	46	365.6	6.111	109.611	46.996	95.005	46.193	57.305	60.32
	47	338.6	6.111	109.611	43.933	98.068	43.169	63.123	64.37
	48	332.0	5.912	106.038	47.307	90.065	33.052	65.166	72.35
	49	346.8	6.111	109.611	46.849	95.152	42.170	61.287	64.41
	Average	345.8	6.061	108.718	46.271	94.573	41.146	61.720	65.36
18 Lespedeza hay and soybean oil meal	46	366.5	6.007	106.481	54.310	84.006	33.982	58.527	69.67
	47	342.2	6.007	106.481	62.873	75.443	27.840	55.858	74.04
	48	335.2	6.007	106.481	67.426	70.890	24.493	54.581	76.99
	49	348.8	6.007	106.481	54.178	84.138	29.859	62.605	74.41
	Average	348.2	6.007	106.481	59.697	78.619	29.044	57.893	73.78
15 Soybean oil meal	46	381.5	6.053	90.968	46.200	76.851	33.018	52.480	68.29
	47	349.5	6.053	90.968	49.208	73.843	26.339	55.839	75.62
	48	347.0	6.053	90.968	46.165	76.886	25.255	59.936	77.95
	49	366.5	6.053	90.968	40.178	82.873	36.022	55.354	66.79
	Average	361.1	6.053	90.968	45.438	77.613	30.159	55.902	72.16
23 Late cut lespedeza hay	46	378.1	6.305	134.046	77.847	89.613	61.452	36.771	41.03
	47	360.4	6.305	134.046	81.115	86.345	51.342	43.442	50.31
	48	355.2	6.305	134.046	79.916	87.544	53.234	42.696	48.77
	49	375.1	6.305	134.046	77.316	90.144	57.875	40.850	45.32
	Average	367.2	6.305	134.046	79.048	88.412	55.976	40.940	46.36
22 Lespedeza hay	24	369.9	6.517	129.477	66.984	97.034	40.391	65.175	67.17
	26	379.0	6.517	129.477	64.007	100.011	44.150	64.480	64.47
	27	362.9	6.517	129.477	62.215	101.803	42.712	67.555	66.36
	28	340.4	6.517	129.477	59.414	104.604	39.725	73.114	69.90
	Average	363.1	6.517	129.477	63.155	100.863	41.745	67.581	66.98
20 Lespedeza hay and lespedeza seed	24	376.7	6.504	193.271	70.286	157.454	88.346	77.708	49.35
	26	387.8	6.504	193.271	66.517	161.223	92.261	77.666	48.17
	27	376.5	6.504	193.271	73.583	154.157	96.042	66.715	43.28
	28	350.0	6.504	193.271	71.698	156.042	84.028	80.349	51.49
	Average	372.8	6.504	193.271	70.521	157.219	90.169	75.610	48.07
21 Lespedeza hay and lespedeza seed	46	376.5	6.066	194.319	71.147	155.320	106.722	57.193	36.82
	47	361.5	6.066	194.319	72.299	154.168	92.473	70.144	45.40
	48	353.8	6.066	194.319	78.706	147.761	99.705	56.429	38.19
	49	368.3	6.066	194.319	75.581	150.886	93.793	65.609	43.48
	Average	365.0	6.066	194.319	74.433	152.034	98.173	62.343	40.97

TABLE 9

COMPARISON OF FECAL METABOLIC NITROGEN, ENDOGENOUS URINARY NITROGEN, RETAINED NITROGEN, AND BIOLOGICAL VALUES COMPUTED FROM INDIVIDUAL AND AVERAGE FORMULA

Ration	Heifer No.	Fecal Metabolic Nitrogen		Endogenous Urinary Nitrogen		Retained Nitrogen		Biological Values	
		Individual	Average	Individual	Average	Individual	Average	Individual	Average
		gm.	gm.	gm.	gm.	gm.	gm.	%	%
2 Alfalfa hay	3	32.322	32.855	7.956	8.657	64.024	65.258	72.27	73.23
	5	37.597	32.855	6.276	8.619	81.471	79.072	81.10	82.61
	6	31.735	31.758	6.774	8.444	73.114	74.834	78.02	79.81
	7	33.654	32.855	9.789	8.685	84.388	82.485	84.07	82.83
	Mean							78.87	79.62
	S. E.*							2.504	2.238
3 Lespedeza hay	3	32.452	32.987	8.230	8.779	70.095	71.179	86.34	87.10
	5	37.749	32.987	6.501	8.751	79.281	76.769	88.12	90.10
	6_1	31.866	31.890	6.553	8.335	70.690	72.496	94.74	97.13
	6_2	31.866	31.890	7.004	8.561	66.078	67.659	87.22	89.27
	7_1	32.666	31.890	9.271	8.484	76.486	74.923	99.08	98.04
	7_2	33.790	32.987	10.244	8.844	75.837	73.634	94.88	93.06
	Mean							91.73	92.45
	S. E.							2.125	1.822
4 Lespedeza hay and corn	3	31.983	32.509	8.394	8.853	67.869	68.854	83.17	83.84
	5	37.203	32.509	6.528	8.761	69.465	67.004	84.22	86.14
	6	31.400	31.424	7.178	8.657	65.672	67.175	87.40	89.37
	7	33.302	32.509	10.751	9.026	77.793	75.275	96.23	94.04
	Mean							87.76	88.35
	S. E.							2.940	2.194
5 Corn	3	32.108	32.635	8.705	8.990	61.684	62.496	69.47	69.97
	5	37.348	32.635	6.805	8.917	76.686	74.089	83.08	84.59
	Mean							76.28	77.28
	S. E.							6.777	7.310
7 Lespedeza hay	24	33.230	34.238	8.303	8.122	60.736	61.563	86.40	86.34
	26	33.558	32.815	8.155	8.132	62.219	61.453	89.43	89.28
	27	34.333	34.926	8.000	8.006	64.829	65.428	87.58	87.68
	28	30.504	34.926	9.391	7.846	68.397	71.274	90.88	89.44
	Mean							88.57	88.19
	S. E.							1.027	0.624
8 Lespedeza hay	24	32.745	32.653	8.157	8.111	63.841	63.703	82.08	82.00
	26	33.546	32.653	8.129	8.153	69.146	68.277	89.54	89.45
	27	32.936	32.653	8.000	8.049	63.841	63.607	81.80	81.80
	28	29.055	32.653	9.039	7.813	71.294	73.666	84.46	83.70
	Mean							84.47	84.24
	S. E.							1.792	1.769
9 Alfalfa hay	24	33.737	32.599	8.684	8.375	66.181	64.734	72.61	71.92
	26	33.638	32.599	8.517	8.345	59.857	58.646	70.84	70.27
	27	33.718	32.599	8.184	8.173	64.541	63.411	75.64	75.30
	28	29.542	32.599	9.194	7.996	63.798	65.657	74.58	74.10
	Mean							73.42	72.90
	S. E.							1.035	1.093
10 Lespedeza hay and milk	24	35.039	32.834	8.663	8.415	71.714	62.261	82.37	81.62
	26	34.035	32.834	8.627	8.425	72.204	70.791	82.29	81.80
	27	34.804	32.834	8.342	8.275	73.743	71.706	86.03	85.62
	28	30.294	32.834	9.107	8.070	71.490	72.993	85.74	84.96
	Mean							84.11	83.50
	S. E.							0.993	1.043
11 Milk	24	35.857	32.614	8.779	8.513	66.388	62.879	67.81	66.42
	26	33.961	32.614	8.854	8.552	67.000	65.351	66.28	65.52
	27	35.407	32.614	8.646	8.444	71.840	68.845	73.57	72.58
	28	30.626	32.614	9.241	8.245	63.969	64.961	73.32	72.80
	Mean							70.25	69.33
	S. E.							1.843	1.949
12 Lespedeza seed	24	38.229	32.844	8.498	8.493	67.990	62.600	72.79	71.12
	26	34.511	32.844	8.822	8.609	64.517	62.637	69.45	68.66
	27	37.343	32.844	8.473	8.464	67.401	64.893	75.88	74.63
	28	31.921	32.844	8.606	8.245	70.644	71.206	77.98	77.81
	Mean							74.03	73.06
	S. E.							1.827	1.973
22 Lespedeza hay	24	41.313	34.541	8.460	8.532	71.875	65.175	69.24	67.17
	26	36.451	34.541	8.747	8.619	66.518	64.480	65.26	64.47
	27	40.159	34.541	8.353	8.464	73.062	67.555	68.02	66.36
	28	34.137	34.541	8.242	8.235	72.717	73.114	69.79	69.90
	Mean							68.08	66.98
	S. E.							0.981	1.077
20 Lespedeza hay and seed	24	42.338	34.469	8.487	8.600	85.464	77.708	51.70	49.35
	26	36.537	34.469	8.862	8.704	79.892	77.666	48.93	48.17
	27	40.959	34.469	8.550	8.600	73.155	66.715	45.54	43.28
	28	34.631	34.469	8.136	8.335	80.312	80.349	51.42	51.49
	Mean							49.40	48.07
	S. E.							1.414	1.750

*Standard error of the mean.

Effect of Gestation on Biological Values.—Heifer 7 consistently gave biological values much higher than the other three heifers fed the same rations. Near the close of the experiments with this group of heifers it became apparent that heifer 7 was well along in gestation. This fact is udoubtedly the explanation of her more economical use of protein. In order to render the values secured in the first experiment comparable with those of later experiments when the protein was used for growth and maintenance only, the results for heifer 7 were not included in the averages in Tables 8, 10 and 11. Comparing the data for heifer 7 with the averages from Table 8 for each ration she was fed thus indicates how much more efficiently she used the feed protein. Her biological value margins over the other three heifers were about 4.3 per cent on the alfalfa ration, 4.2 and 9.2 per cent on the lespedeza ration and 7.6 per cent on the corn and lespedeza ration.

Effect of Order of Feeding on Biological Values.—In the plan of the first experiments the order of feeding the rations was different for each pair of heifers, as shown in Table 7. The effect of these differences in order upon the biological values can be seen in the results (Table 8) secured with each pair. The variations secured do not appear to be connected with the order of feeding. The difference between lespedeza and alfalfa biological values were practically the same for each pair although one received alfalfa immediately following the low-protein ration and the other received lespedeza. The differences between the biological values from the lespedeza ration and from the corn and lespedeza ration are likewise unexplainable from the standpoint of order of feeding. Unfortunately the composite sample of urine of heifer 6 for her first lespedeza feeding period was spilled during filtration; so the comparison of effect of order is not complete. The only evidence obtained, and it was slight, of a higher utilization of nitrogen during the first period following the low-nitrogen feeding as compared to later periods was in case of heifer 7. The first lespedeza feeding for this heifer gave a biological value of 98.04 and the second period, forty days later, gave a biological value of 93.06. This difference is hardly significant however; and it may be further explained by the fact that during the second period her daily feed was increased from 14.5 to 15 pounds. Consequently she had more absorbed nitrogen available for utilization, yet no more was utilized. The inverse relationship between biological value and absorbed nitrogen for dairy heifers will be discussed later.

Although the number of determinations involved was small, the effect of changes in the order of feeding the rations seemed so small in the first trial as to be almost negligible. Hence, for the sake of simplicity in feeding and handling the rations, in subsequent trials the order of feeding was kept the same for all four heifers.

TABLE 10

INDIVIDUAL DATA AND RATION AVERAGES OF PROTEIN NUTRITIVE VALUE

Ration	Heifer No.	Nitrogen Balance	Utilized Nitrogen	True Digestibility	Apparent Digestibility	Biological Value	Apparent Net Protein Value	Nitrogen Intake Stored	Utilized Nitrogen Stored	Digested Nitrogen Stored
		gm./day	gm./day	%	%	%	%	%	%	%
2 Alfalfa hay	3	23.75	65.26	79.53	50.21	73.23	36.77	21.19	36.39	42.20
	5	37.60	79.07	85.42	56.10	82.61	46.34	33.55	47.55	59.81
	6	34.61	74.83	86.56	57.21	79.81	45.66	31.95	46.25	55.84
	7**	40.95	82.49	88.86	59.55	82.83	49.33	36.87	49.64	61.36
	Mean	31.99	73.05	83.84	54.51	78.55	42.92	28.90	43.40	52.62
	S. E*	4.21	4.80	2.15	2.15	2.78	3.16	3.88	3.52	2.65
3 Lespedeza hay	3	29.41	71.18	76.80	45.80	87.10	39.89	27.64	41.32	60.35
	5	35.03	76.77	80.07	49.07	90.10	44.21	32.92	45.63	67.09
	6_1**			72.56	41.56					
	6_2	27.21	67.66	73.68	42.68	89.27	38.10	26.45	40.22	61.98
	7_1**	34.55	74.92	74.29	43.29	98.04	42.44	33.59	46.12	77.58
	7_2**	31.80	73.63	74.36	43.36	93.06	40.35	29.89	43.19	68.93
	Mean	30.55	71.87	76.85	45.85	88.82	40.73	29.00	42.39	63.14
	S. E.	2.33	2.65	1.85	1.85	0.97	1.81	1.99	1.65	2.03
4 Lespedeza hay and corn	3	27.49	68.85	75.21	45.44	83.84	38.10	25.18	39.93	55.41
	5	25.73	67.00	71.23	41.46	86.14	35.71	23.57	38.40	56.84
	6	27.09	67.18	71.21	41.44	89.37	37.04	25.67	40.32	61.94
	7**	33.74	75.28	73.30	43.53	94.04	40.94	30.90	44.82	70.98
	Mean	26.77	67.68	72.55	42.78	86.45	36.95	24.81	39.55	58.06
	S. E.	0.53	0.59	1.33	1.33	1.60	0.69	0.63	0.59	2.01
5 Corn	3	20.87	62.50	87.22	55.35	69.97	38.73	20.38	33.39	36.82
	5	32.53	74.09	85.53	53.63	84.59	45.37	31.77	43.91	59.20
	Mean	26.70	68.30	86.38	54.49	77.28	42.05	26.08	38.65	48.01
	S. E.	5.83	5.80	0.53	0.86	7.31	3.32	5.70	5.26	4.57
7 Lespedeza hay	24	19.20	61.56	71.00	36.91	86.34	31.87	19.12	31.19	51.81
	26	20.51	61.45	71.78	37.56	89.28	33.53	21.39	33.38	56.94
	27	22.50	65.43	72.92	38.79	87.68	34.01	21.98	34.39	56.68
	28	28.50	71.27	77.87	43.74	89.44	39.12	27.85	39.99	63.68
	Mean	22.68	64.93	73.39	39.25	88.19	34.62	22.59	34.74	57.28
	S. E.	2.06	2.31	1.56	1.55	0.62	1.57	1.86	1.87	2.43
8 Lespedeza hay	24	22.94	63.70	71.89	41.68	82.00	34.18	21.23	36.01	50.93
	26	27.47	68.28	70.63	40.42	89.45	36.16	25.42	40.23	62.90
	27	22.91	63.61	71.96	41.74	81.80	34.14	21.20	36.02	50.78
	28	33.20	73.67	81.45	51.23	83.70	42.88	30.72	45.07	59.97
	Mean	26.63	67.32	73.98	43.77	84.24	36.84	24.64	39.33	56.15
	S. E.	2.44	2.38	2.52	2.50	1.77	2.07	2.26	2.16	3.10
9 Alfalfa hay	24	23.76	64.73	83.88	53.50	71.92	38.48	22.14	36.71	41.39
	26	17.70	58.65	77.79	47.40	70.27	33.31	16.50	30.18	34.80
	27	22.64	63.41	78.48	48.10	75.30	36.22	21.10	35.70	43.87
	28	25.06	65.66	82.57	52.20	74.10	38.68	23.36	38.17	44.75
	Mean	22.29	63.11	80.68	50.30	72.90	36.67	20.78	35.19	41.20
	S. E.	1.61	1.56	1.50	1.50	1.09	1.25	1.50	1.75	2.26
10 Lespedeza hay and milk	24	28.01	69.26	77.80	47.70	81.62	38.93	25.68	40.44	53.85
	26	29.53	70.79	79.35	49.25	81.80	40.29	27.08	41.72	54.98
	27	30.60	71.71	76.78	46.68	85.62	39.97	28.05	42.67	60.10
	28	32.09	72.99	78.77	48.67	84.96	41.35	29.42	43.97	60.45
	Mean	30.06	71.19	78.18	48.08	83.50	40.14	27.56	42.20	57.35
	S. E.	0.83	0.79	0.43	0.49	1.04	0.50	0.79	0.75	1.68
11 Milk	24	21.75	62.88	85.83	56.26	66.42	37.37	19.72	34.59	35.06
	26	24.19	65.35	90.44	60.87	65.52	39.88	21.93	37.02	36.03
	27	27.79	68.85	86.00	56.43	72.58	40.96	25.19	40.36	44.65
	28	24.10	64.96	80.91	51.34	72.80	37.38	21.85	37.10	42.57
	Mean	24.46	65.51	85.80	56.23	69.33	38.90	22.17	37.27	39.58
	S. E.	1.25	1.24	1.91	1.92	1.95	0.91	1.13	1.18	2.37
12 Lespedeza seed	24	21.26	62.60	80.36	50.37	71.12	35.82	19.41	33.96	38.53
	26	21.18	62.64	83.28	53.30	68.66	36.60	19.34	33.81	36.29
	27	23.59	64.89	79.38	49.40	74.63	36.87	21.53	36.35	43.59
	28	30.12	71.21	83.54	53.56	77.81	41.68	27.49	42.30	51.33
	Mean	24.04	65.34	81.64	51.66	73.06	37.74	21.94	36.61	42.44
	S. E.	2.10	2.03	1.04	1.02	1.97	1.33	1.92	1.99	3.32

*Standard error of the mean.

**Records of these periods were not included in the averages of the respective rations.

TABLE 10 (continued)

INDIVIDUAL DATA AND RATION AVERAGES OF PROTEIN NUTRITIVE VALUE

Ration	Heifer No.	Nitrogen Balance gm./day	Utilized Nitrogen gm./day	True Digestibility %	Apparent Digestibility %	Biological Value %	Apparent Net Protein Value %	Nitrogen Intake Stored %	Utilized Nitrogen Stored %	Digested Nitrogen Stored %
16 Lespedeza hay	46	22.63	63.35	75.02	43.50	81.55	35.47	21.85	35.72	50.23
	47	22.82	63.26	66.59	35.08	91.73	32.18	22.03	36.07	62.81
	48	23.98	64.31	72.53	41.01	85.62	35.11	23.16	37.29	56.46
	49	23.09	63.55	76.36	44.85	80.36	36.04	22.30	36.33	49.72
	Mean	23.13	63.62	72.63	41.11	84.82	34.70	22.34	36.35	54.81
	S. E.	0.30	0.24	2.13	2.16	2.54	0.86	0.29	0.34	3.06
14 Soybean oil meal	46	36.29	76.88	89.42	60.00	78.15	46.89	32.99	47.20	54.98
	47	34.95	75.27	87.03	57.62	78.61	45.30	31.77	46.43	55.14
	48	35.07	75.29	83.88	54.47	81.58	44.44	31.87	46.59	58.52
	49	30.85	71.23	84.70	55.29	76.44	42.26	30.85	43.31	50.72
	Mean	34.29	74.67	86.26	56.85	78.70	44.72	31.87	45.88	54.84
	S. E.	1.19	1.21	1.22	1.21	1.01	0.97	0.44	0.87	1.60
17 Lespedeza hay and milk	46	27.43	68.22	78.60	48.84	79.73	38.94	25.20	40.21	51.60
	47	28.24	68.72	75.05	45.28	84.12	38.09	25.94	41.09	57.29
	48	27.44	67.88	75.67	45.90	82.40	37.82	25.20	40.42	54.91
	49	26.28	66.84	77.90	48.14	78.82	37.94	24.14	39.32	50.14
	Mean	27.35	67.92	76.81	47.04	81.27	38.20	25.12	40.26	53.49
	S. E.	1.28	0.40	0.78	0.86	1.19	0.25	0.37	0.37	1.59
19 Milk	46	16.42	57.31	86.68	57.13	60.32	34.46	14.98	28.65	26.23
	47	22.51	63.12	89.47	59.92	64.37	38.57	20.54	35.66	34.27
	48	25.68	65.17	84.94	55.39	72.35	40.08	24.22	39.41	43.72
	49	20.59	61.29	86.81	57.26	64.41	36.88	18.79	33.59	32.81
	Mean	21.30	61.72	86.98	57.43	65.36	37.50	19.63	34.33	34.26
	S. E.	1.94	1.67	0.85	0.88	2.53	1.21	1.92	2.24	3.60
18 Lespedeza hay and soybean oil meal	46	18.19	58.53	78.89	49.00	69.67	34.14	17.08	31.08	34.86
	47	15.77	55.86	70.85	40.95	74.04	30.32	14.81	28.23	36.16
	48	14.56	54.58	66.58	36.68	76.99	28.24	13.68	26.68	37.29
	49	22.44	62.61	79.02	49.12	74.41	36.55	21.08	35.84	42.91
	Mean	17.74	57.90	73.84	43.94	73.78	32.31	16.66	30.46	37.81
	S. E.	1.74	1.77	3.06	3.08	1.50	1.87	1.63	2.01	1.75
15 Soybean oil meal	46	11.75	52.48	84.48	49.21	68.29	33.61	12.92	22.39	26.25
	47	15.42	55.84	81.18	45.91	75.62	34.72	16.95	27.62	36.93
	48	19.55	59.94	84.52	49.25	77.95	38.39	21.49	32.62	43.63
	49	14.77	55.35	91.10	55.83	66.79	37.29	16.23	26.69	29.08
	Mean	15.37	55.90	85.32	50.05	72.16	36.00	16.90	27.33	33.97
	S. E.	1.61	1.54	2.08	2.08	2.74	1.11	1.76	2.10	3.94
23 Late cut lespedeza hay alone	46	-5.25	36.77	66.85	41.93	41.03	17.20	-3.92	-14.28	-9.35
	47	+1.59	43.44	64.41	39.49	50.31	19.87	+1.19	+3.66	+3.00
	48	+0.90	42.70	65.31	40.38	48.77	19.69	+0.67	+2.11	+1.66
	49	-1.15	40.85	67.25	42.32	45.32	19.18	-0.86	-2.82	-2.02
	Mean	-0.98	40.94	65.96	41.03	46.36	18.99	-0.73	-2.83	-1.68
	S. E.	1.54	1.49	0.58	0.66	2.10	0.59	1.15	4.06	2.77
22 Lespedeza hay alone	24	22.10	65.18	74.94	48.27	67.17	32.42	17.07	33.91	35.37
	26	21.32	64.48	77.24	50.57	64.47	32.60	16.47	33.07	32.56
	27	24.55	67.56	78.62	51.95	66.36	34.47	18.96	36.34	36.50
	28	30.34	73.11	80.79	45.89	69.90	32.08	23.43	41.50	43.30
	Mean	24.58	67.58	77.90	49.17	66.98	32.89	18.98	36.21	36.93
	S. E.	2.04	1.96	1.20	1.33	1.08	0.54	1.58	1.90	2.29
20 Lespedeza hay and seed	24	34.64	77.71	81.47	63.63	49.35	31.40	17.92	44.58	28.17
	26	34.49	77.67	83.42	65.58	48.17	31.59	17.85	44.41	27.21
	27	21.65	66.72	79.76	61.93	43.28	26.80	11.20	32.45	18.09
	28	37.55	80.35	80.74	62.90	51.49	32.39	19.43	46.73	30.89
	Mean	32.08	75.61	81.35	63.51	48.07	30.55	16.60	42.04	26.09
	S. E.	3.55	3.04	0.73	0.77	1.75	1.25	1.84	3.25	2.78
21 Late cut lespedeza hay and seed	46	16.45	57.19	79.93	63.39	36.82	23.34	8.47	28.76	13.36
	47	29.55	70.14	79.34	62.79	45.40	28.51	15.21	42.13	24.22
	48	15.91	56.43	76.04	59.50	38.19	22.72	8.19	28.19	13.76
	49	24.95	65.61	77.65	61.11	43.48	26.57	12.84	38.03	21.01
	Mean	21.72	62.34	78.24	61.70	40.97	25.29	11.18	34.28	18.09
	S. E.	3.33	3.33	0.88	0.88	2.06	1.37	1.71	3.46	2.70

TABLE 11

SUMMARY OF RATION AVERAGES OF MEASURES OF NITROGEN UTILIZATION AND PROTEIN NUTRITIVE VALUE

Ration No.	Nitrogen Balance	Utilized Nitrogen	True Digestibility	Apparent Digestibility	Biological Value	True Net Protein Value	Apparent Net Protein Value	Nitrogen Intake Stored	Utilized Nitrogen Stored	Digested Nitrogen Stored
	gm./day	gm./day	%	%	%	%	%	%	%	%
2	31.99	73.05	83.84	54.51	78.55	3.76	42.92	28.90	43.40	52.62
3	30.55	71.87	76.85	45.85	88.82	3.65	40.73	29.00	42.39	63.14
4	26.77	67.68	72.55	42.78	86.45	3.31	36.95	24.81	39.55	58.06
5	26.70	68.30	86.38	54.49	77.28	3.29	42.05	26.08	38.65	48.01
7	22.68	64.93	73.39	39.25	88.19	2.70	34.62	22.59	34.74	57.28
8	26.63	67.32	73.98	43.77	84.24	3.19	36.84	24.64	39.33	56.15
9	22.29	63.11	80.68	50.30	72.90	2.81	36.67	20.78	35.19	41.20
10	30.06	71.19	78.18	48.08	83.50	3.54	40.14	27.56	42.20	57.35
11	24.46	65.51	85.80	56.23	69.33	3.03	38.90	22.17	37.27	39.58
12	24.04	65.34	81.64	51.66	73.06	2.99	37.74	21.94	36.61	42.44
16	23.13	63.62	72.63	41.11	84.82	2.86	34.70	22.34	36.35	54.81
14	34.29	74.67	86.26	56.85	78.70	3.89	44.72	31.87	45.88	54.84
17	27.35	67.92	76.81	47.04	81.27	3.27	38.20	25.12	40.26	53.49
19	21.30	61.72	86.98	57.43	65.36	2.75	37.50	19.63	34.33	34.26
18	17.74	57.90	73.84	43.94	73.78	2.41	32.31	16.66	30.46	37.81
15	15.37	55.90	85.32	50.05	72.16	2.20	36.00	16.90	27.33	33.97
					Averages					
Alfalfa	26.45	67.37	82.03	52.10	75.32	3.29	39.35	24.26	38.71	46.09
Lespedeza	25.43	66.60	74.04	42.27	86.36	3.10	36.45	24.35	37.92	57.49
Milk and lespedeza	28.70	69.55	77.49	47.56	82.38	3.40	39.17	26.34	41.23	55.42
Milk	22.88	63.62	87.64	56.83	67.35	2.99	38.20	20.90	35.80	36.92
Soybean oil meal	24.83	65.29	85.79	53.45	75.43	3.05	40.36	24.39	36.61	44.41
					Digestibility Rations					
20	32.08	75.61	81.35	63.51	48.07	3.54	30.55	16.60	42.04	26.09
21	21.72	62.34	78.24	61.70	40.97	2.77	25.29	11.18	34.28	18.09
22	24.58	67.58	77.90	49.17	66.98	2.85	32.89	18.98	36.21	36.93
23	-0.98	40.94	65.96	41.03	46.36	0.65	18.99	-0.73	-2.83	-1.68

Biological Values of Lespedeza Hay Protein Compared With Alfalfa Hay Protein.—Higher biological values were secured with the first group of heifers than with the others, but the comparisons between similar rations gave similar results for all groups. With the first group the average biological value for alfalfa was 78.55 while that for lespedeza was 88.82. This was a difference of more than 10 per cent. With the second group the biological values for similar rations were 72.90 for alfalfa and 84.24 for lespedeza which gave a difference of 11.34 per cent. The high average biological value of 88.19 secured from ration 7 was probably due largely to its low crude protein content of only 8.82 per cent. Lespedeza hay fed in the third group of experiments at a level of 9.50 per cent protein gave a biological value of 84.82. The average biological value of all of the lespedeza rations in which starch or sugar was fed was 86.36 and the average of all of the alfalfa rations was 75.32. The absorbed nitrogen from the lespedeza rations was undoubtedly utilized more efficiently than that from the alfalfa rations.

Biological Values of Corn and Lespedeza Hay Proteins.—Corn, milk and expeller process soybean oil meal were studied for possible supplementary effects of their proteins with lespedeza hay proteins. Two heifers from the first group gave an average biological value of 77.28 for a ration in which about three-fourths of the protein came from corn gluten meal and the rest came from yellow corn. The average biological value of corn and lespedeza hay was 86.45. Yellow corn and lespedeza furnished protein in this ration at a ratio of 1:6.09. If there were no supplementary effect between these proteins a biological value of 87.19 should have resulted. The evidence secured with the few heifers used is clearly against the existence of a supplementary effect between proteins of lespdeza hay and the corn grain. Since Turk, Morrison and Maynard (1934) had found that neither alfalfa nor clover proteins exhibited supplementary action with corn proteins when fed to sheep and the results secured here indicated not supplementation, it appears that the proteins of the legumes are not enhanced by corn proteins for ruminants.

Biological Values of Dried Skimmilk and Lespedeza Hay Protein.—The possible supplementary effect between milk protein and lespedeza hay protein for growing dairy cattle was of interest because of the common use of these feeds together in calf rearing. Accordingly, in the second trial the average biological value of dried skimmilk protein was determined as 69.33. Lespedeza and milk gave an average biological value of 83.50. The protein of this ration came from milk and lespedeza in the ratio of 1:2.0. If no supplementary effect had existed the expected biological value would have been 79.27 (using the biological value of ration 8 lespedeza hay) or 81.90 (using the biological value of ration 7 lespedeza hay). These results could have been interpreted as a slight supplementary effect, but it was felt that repetition of the experiment might give more definite results. The third group of heifers gave an average biological value for milk of 65.36, and their average biological value for lespedeza was 84.82. The lespedeza and milk ration in which the ratio of milk to lespedeza protein was 1:1.96 gave an average biological value of 81.27. If no supplementary effect had existed the expected biological value for the mixture would have been 78.25. The results of the previous trial were thus confirmed. Taking the eight determinations together, the average biological value was 82.38 and the average expected if no supplementation occurred was 78.76. The mean difference of 3.62 per cent was of low significance statistically. According to Fisher's t test the probability of its occurring as a result of chance was greater than 0.02. It was concluded, therefore, that there was not an important supplementary action between milk proteins and lespedeza proteins.

The average biological value for the milk rations from all eight determinations was 67.35.

Biological Values of Soybean Oil Meal and Lespedeza Hay Proteins.—In an attempt to determine whether or not soybean oil meal protein and lespedeza hay protein were supplementary, rations were fed similar to the milk and lespedeza rations. The results with soybean oil meal in the ration were not consistent in any way with any logically expected interaction. A ration containing soybean oil meal and lespedeza hay in which the crude protein was furnished at the ratio of 1 part soybean to 1:90 parts of lespedeza protein gave an average biological value of 73.78. The lespedeza hay protein at a 9.51 per cent level had given an average biological value of 84.82. Soybean protein at a 10.11 per cent level had given an average biological value of 78.70. Thus the mixture of soybean and lespedeza protein was much lower than either one of them alone. To further complicate a reasonable interpretation of these results, when the soybean oil meal ration was compounded to contain only 8.36 per cent crude protein, the average biological value was 72.16. This was 6.54 per cent lower than the value from the 10.11 per cent protein ration. From previous experience and from the experience of other investigators with many species, it was expected that as the protein percentage of the ration was decreased the biological value would have increased and vice versa; but such was not the case with these soybean oil meal rations. For some unexplainable reason the heifers stored more than twice as much nitrogen on the 10.11 per cent protein ration as they did on the 8.36 per cent ration although the rations were nearly the same in composition. This was probably the appearance of the unusual variation likely to accompany short nitrogen balance experiments which was warned against by Hart, Humphrey and Morrison (1914).

The results from the two soybean oil meal rations averaged together gave a biological value of 75.43 which is probably more comparable with biological values secured from the other rations than is the value of 78.70 for the 10.11 per cent protein ration.

Biological Value of Lespedeza Seed Protein.—Ground Korean lespedeza seed was fed to the four heifers in the second group in a ration in which it furnished nearly 10 per cent protein. The average biological value from this ration was 73.06.

Biological Values in Rations Not Adjusted to Ten Per Cent Crude Protein.—The average biological value of intermediate cut lespedeza hay fed alone, the crude protein content being 11.15 per cent, was 66.98. A ration of solely late-cut lespedeza hay furnishing 11.54 per cent protein gave an average biological value of 46.36. These rations were fed to determine coefficients of digestibility of the hays cut at different stages and the biological values secured are not comparable with those of the lespedeza hay rations to which starch, sugar, grain or other concentrated feeds were added because of the lower supply of energy secured from the hay alone. A ration containing lespedeza seed and intermediate cut lespedeza hay which furnished 16.65 per cent crude protein gave an average biological value of 48.07. This low value was to be expected because of the high content of crude protein in the ration. A similar ration containing late-cut lespedeza hay and lespedeza seed gave an average biological value of 40.97. This ration was also high in crude protein, containing 17.85 per cent.

Combination of Measures of Protein Nutritive Value

The biological values of the various proteins and protein mixtures gave the amount of absorbed nitrogen which the heifers utilized for growth and maintenance. The percentage of the protein which was absorbed is also an important factor in evaluating the nutritive value of the proteins. Also of importance is the actual amount of nitrogen stored on any ration. All of these factors must be considered in the evaluation of feed proteins. If only the biological values were considered, the proteins fed at an 8.5 to 10 per cent level would have ranked as follows: lespedeza, corn and lespedeza, milk and lespedeza, alfalfa, corn, soybean oil meal, soybean oil meal and lespedeza, lespedeza seed, and milk. The same proteins ranked in order of digestibility as follows: milk, soybean oil meal, corn, lespedeza seed, alfalfa, milk and lespedeza, soybean oil meal and lespedeza, corn and lespedeza, and lespedeza hay. It is clear that ranking the proteins according to one system put them almost inversely as they were ranked according to the other; hence neither measure alone can be taken as an index of the net nutritive value of the proteins.

In order to show the relationship of the various methods used in expressing the nutritive value of proteins, the data for individual heifers for all the rations were listed in Table 10. The averages of Table 10 were summarized in Table 11 along with ration averages for those proteins fed more than once. The data for heifer 7 were omitted from the averages for reasons previously set forth. The utilized nitrogen of Tables 10 and 11 is the same as the retained nitrogen of Tables 8 and 9. This change was made so as to not confuse retained and stored nitrogen, the latter being the actual amount of nitrogen stored in the body as indicated by the nitrogen balance. The apparent net protein value is the product of the apparent digestibility times the biological value expressed in per cent. It is merely intended to be a rough estimate of the net value of the protein and should not be confused with the true net protein value, which will be discussed later. From the data presented in Table 10 the differences among rations for the nitrogen balance, utilized nitrogen, apparent net protein value, percentage of nitrogen intake stored, and percentage of utilized nitrogen stored appear to be quite small. In order to test the significance of the differences the data were treated statistically by the analysis of variance of the differences among group means, using the F ratio as presented by

Snedecor (1940) as a measure of significance. Only the rations which were adjusted to a level of 10 per cent protein or lower by the addition of starch and sugar were used for this comparison.

It was found that when the data for all heifers were treated together the differences among the means of the rations were significant for each of the values: nitrogen balance, utilized nitrogen, apparent net protein, percentage of nitrogen intake stored, and percentage of utilized nitrogen stored. The probability of the differences occurring as a result of chance was less than 0.01 in each comparison. Dividing the data according to heifer groups revealed, however, that most of the variation was due to the third group.

The differences among the rations fed to heifers 3, 5 and 6 were not significant for the values listed above; they were less in most cases than the differences among heifers on the same ration. The differences among the means of the rations fed to heifers 24, 26, 27 and 28 for the values listed above were not significant. In all cases the probabilities of the variations observed occurring as a result of chance were more than 0.05. These rations included the proteins of lespedeza hay, alfalfa hay, corn, milk, lespedeza seed, and mixtures of corn with lespedeza hay and milk with lespedeza hay. The utilization of this variety of proteins by these growing heifers was not affected by the kind of protein in rations which were equalized as to protein content and fed at a constant level.

The results secured with the third group of heifers were in accord with the previous observations for rations containing lespedeza hay, milk, or milk and lespedeza hay; but rations which contained soybean oil meal or soybean oil meal and lespedeza hay were at considerable variance with the others. Furthermore, the results from the soybean oil meal rations were not consistent. The nitrogen utilization during one soybean feeding period was the highest of the entire experiment and during the other periods it was the lowest. It is not logical to explain such extremes as being due to the kind of protein fed. Hence, it is believed that the theory of lack of difference between the utilization of proteins as borne out by the majority of the experiments should still be tenable.

The amount of nitrogen stored from these rations which furnished about the same energy intake and nearly 100 grams of nitrogen daily was about 25 grams per day (see Table 11). The stored nitrogen as percentage of the nitrogen intake was therefore about 25 per cent. The amount of nitrogen utilized daily by the heifers for storage and maintenance, the latter including fecal metabolic and endogenous urinary nitrogen, was approximately 65 to 70 grams of which only about three-eighths was stored. These values were approximately repeated in experiment after experiment regardless of the source of the protein. The digestion coefficients show, also, that the amount of nitrogen absorbed was widely different for the different rations. The apparent digestibility of crude protein varied from about 40 per cent for lespedeza to 55 per cent or more for corn, alfalfa, and milk. The true digestibility varied in a similar manner. The result of this variation in supply of utilizable (absorbed) nitrogen and relative constancy of amount utilized was biological values (per cent of absorbed nitrogen utilized) which varied inversely as absorption. The apparent net protein value (biological value times per cent digestible nitrogen) should therefore give a fairly accurate index for comparing the net efficiency of the proteins of various rations. As stated above the apparent net protein values were not significantly different for different rations fed to the same heifers in the first two trials. Furthermore, if the extraordinarily high value for ration 14 is omitted from the comparisons for the third group of heifers, the differences between the apparent net protein values of the remaining rations are of only slight significance.

Since the amount of nitrogen utilized or stored was fairly constant and the digestibility of the proteins was quite variable, it is clear that the per cent of digested nitrogen stored should be expected to be very variable. The columns on the extreme right of Tables 10 and 11 give these percentages. These values, although quite widely used in the literature, give no idea of the net worth of the protein and are of little use for comparative purposes.

While the apparent net protein value gave a better index of the comparative nutritive value of proteins than either the biological value or the coefficient of apparent digestibility alone, it can not be used to estimate accurately the amount of nitrogen which will be stored from a ration. A demonstration of this fact is given by the data in Table 12. The apparent net protein value times the daily nitrogen intake gives the amount of net nitrogen which should be available for growth and maintenance—the latter expressed by the urinary endogenous nitrogen. A comparison of the results of this calculation, the apparent net nitrogen, with the actual amount used for nitrogen storage and endogenous urinary nitrogen shows that the former gives values which are always too high. The apparent net protein value can not be used to estimate nitrogen utilization accurately because the biological value is derived from the total nitrogen absorbed or true digestibility rather than the apparent digestibility which was used in calculating the apparent net protein value.

When the true net protein value of the ration was calculated and multiplied by the amount of the ration consumed then divided by 6.25, the amount of true net nitrogen was secured. These values were practically the same as the actual nitrogen balance plus the urinary endogenous nitrogen excretion. The true net protein of the ration would have definite nutritional significance, therefore, if it could be calculated for any ration. It is hardly fair as a means of comparing the efficiency of the proteins of all of the rations in

TABLE 12

COMPARISON OF THE AVERAGE TRUE NET PROTEIN AND APPARENT NET PROTEIN VALUES OF THE RATIONS

Ration No.	Protein	True Net Protein of Ration	Daily Feed Intake	True Net Nitrogen	Nitrogen Balance plus Urine Endogenous Nitrogen	Apparent Net Nitrogen	Daily Nitrogen Intake	Apparent Net Protein	True Net Protein, Per Cent of Total
		%	kg.	gm.	gm.	gm.	gm.	%	%
2	Alfalfa hay	3.76	6.7283	40.48	40.56	47.56	110.815	42.92	36.54
3	Lespedeza hay	3.65	6.7283	39.29	39.25	42.86	105.231	40.73	37.32
4	Lespedeza & corn	3.31	6.7283	35.63	35.53	39.90	107.990	36.95	33.00
5	Corn	3.29	6.8039	35.82	35.65	43.06	102.399	42.05	34.96
7	Lespedeza hay	2.70	7.1103	30.72	30.71	34.70	100.245	34.62	30.61
8	Lespedeza hay	3.19	6.8039	34.73	34.66	39.81	108.062	36.84	32.13
9	Alfalfa hay	2.81	6.8039	30.59	30.51	39.35	107.299	36.67	28.50
10	Milk & lespedeza	3.54	6.8039	38.54	38.36	43.78	109.069	40.14	35.26
11	Milk	3.03	6.8039	32.99	32.90	42.90	110.292	38.90	31.69
12	Lespedeza seed	2.99	6.8039	32.55	32.49	41.34	109.544	37.74	29.69
16	Lespedeza hay	2.86	6.8039	31.14	30.98	35.93	103.555	34.70	30.07
14	Soybean oil meal	3.89	6.8039	42.35	42.30	49.20	110.019	44.72	38.48
17	Milk & lespedeza	3.27	6.8039	35.60	35.52	41.59	108.862	38.20	32.70
19	Milk	2.75	6.7485	29.69	29.59	40.77	108.718	37.50	27.31
18	Soybean and lespedeza hay	2.41	6.8039	26.24	26.05	34.40	106.481	32.31	24.64
15	Soybean oil meal	2.20	6.8039	23.95	23.82	32.75	90.968	36.00	26.32
22	Lespedeza hay	2.85	7.2575	33.09	33.04	42.59	129.477	32.89	25.56
23	Lespedeza hay	0.65	7.2575	7.55	7.52	25.46	134.046	18.99	5.63
20	Lespedeza hay and seed	3.54	7.2575	41.11	40.64	59.04	193.271	30.55	21.28
21	Lespedeza hay and seed	2.77	6.8039	30.16	30.20	49.14	194.319	25.29	15.52

Table 12, however, because there were some important variations in the percentage of protein in the different rations. A figure of more value for comparison is the true net protein expressed as a percentage of the total protein of the ration. These are listed in the last column of Table 12. Although the variations in these values are not always associated with like variations in the apparent net protein values, the two agree in demonstrating only minor variations between rations fed to the same group of heifers.

The poor utilization of nitrogen from ration 23, late-cut lespedeza hay, was due to the very poor digestibility of the hay so that the total digestible nutrient intake was not sufficient to permit growth or nitrogen storage.

Factors Affecting Biological Values for Growing Cattle

Since the biological values of proteins have been used so widely to express their nutritive value, it seemed appropriate to analyze the status of the biological values secured in this study especially from the view of factors affecting them and their place in expressing the nutritive value of proteins.

Concentration of Protein in the Ration.—The low biological values secured with rations 20 and 21 clearly emphasize the often observed fact that as the concentration of protein in the ration is increased the biological value will fall. Another comparison of lespedeza hay proteins fed at different levels was that of rations 7 and 8 which has already been discussed. One comparison, previously mentioned in which the general rule was not followed was that of the soybean oil meal rations, 14 and 15.

Level of Total Nutrient Intake.—The results from feeding the late-cut lespedeza hay clearly demonstrate that nitrogen utilization is secondary to an adequate intake of digestible nutrients. The late-cut lespedeza hay was fed at the same rate, 16 pounds daily, as the early-cut hay of ration 22. Nitrogen equilibrium was not reached on the average with the late-cut hay, while nearly as much nitrogen was stored from the early-cut hay as from the previous rations containing concentrated energy-yielding feeds. The average amount of nitrogen absorbed from the rations did not differ greatly as shown in Table 8. The late-cut hay furnished an average of only 6.07 pounds of total digestible nutrients daily, which was barely more than the maintenance requirements of the heifers; and the early cut hay furnished 8.33 pounds of total digestible nutrients daily (shown in Table 13). This wide difference in nutrient intake was undoubtedly responsible for the difference in efficiency of nitrogen utilization.

Amount of Nitrogen Absorbed.—It was observed (Table 8) that rations from which similar amounts of nitrogen were absorbed had similar biological values; and, furthermore, that the greater the amount of absorbed nitrogen the lower was the biological value. This general relationship prevailed because the utilized nitrogen did not vary significantly from ration to ration, as was previously discussed. The correlation between absorbed nitrogen and biological value is graphically presented in Fig. 10. The all-hay rations and the hay and seed rations were not included in this analysis because of their higher concentration of protein. The correlation coefficient, r, for absorbed nitrogen and biological values was —0.573± 0.083 for all of the rations which were adjusted to approximately 10 per cent protein. When the abnormal data from the two soybean oil meal rations were omitted, the correlation coefficient was —0.716± 0.069.

The last soybean oil meal ration fed was adjusted to a level of about 8.4 per cent protein for the purpose of testing this relationship between biological value and absorbed nitrogen. It was expected that the same amount of nitrogen would be absorbed from that ration as had been absorbed from the lespedeza hay rations and that similar biological values would result. The expectations were achieved in part. An average of 77.613 grams of nitrogen was absorbed daily from ration 15 and the average biological value was 72.16. From ration 18, which contained lespedeza hay, an average of 78.619 grams of nitrogen was absorbed daily and the biological value was 73.78. Ration 18 had been fed just previously to ration 15 with the same group of heifers. Unfortunately, these heifers at that time were behaving abnormally as far as nitrogen storage was

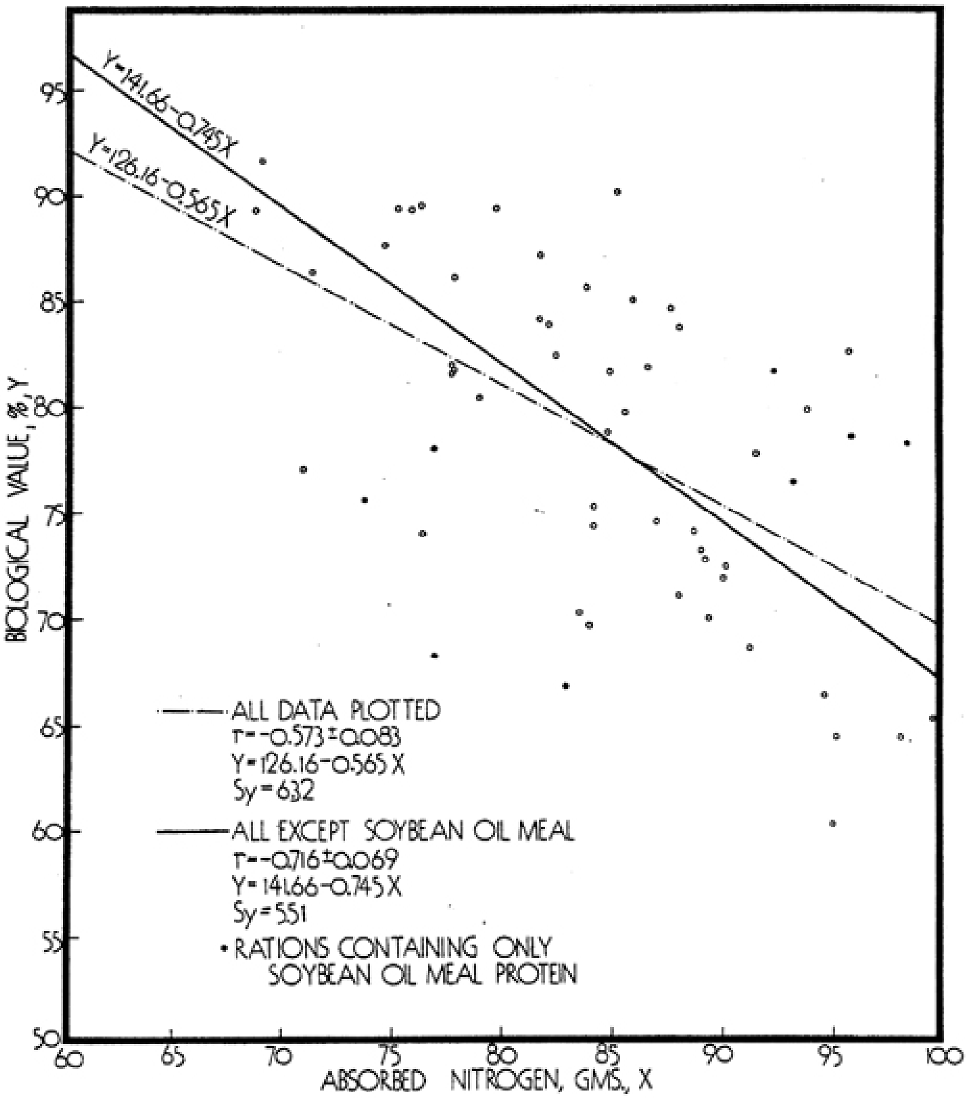


Fig. 10.—Biological values of protein in rations containing about ten per cent crude protein plotted against the amount of nitrogen absorbed daily. The average regression lines fitted to all of the data and to all except the soybean oil meal rations are given on the chart along with the coefficients of correlation and the standard errors of estimate.

concerned; so the comparisons could not be extended beyond those two rations.

Another method of expressing the absorbed nitrogen is the coefficient of true digestibility. The correlation coefficient for biological values and the true digestibility was —0.643± 0.077 for all of the adjusted rations. When the soybean oil meal rations were omitted the corresponding correlation coefficient was —0.680± 0.076.

The most common method of designating the absorbability of a protein is to give the coefficient of apparent digestibility. This term includes as non-absorbable nitrogen much feces nitrogen of

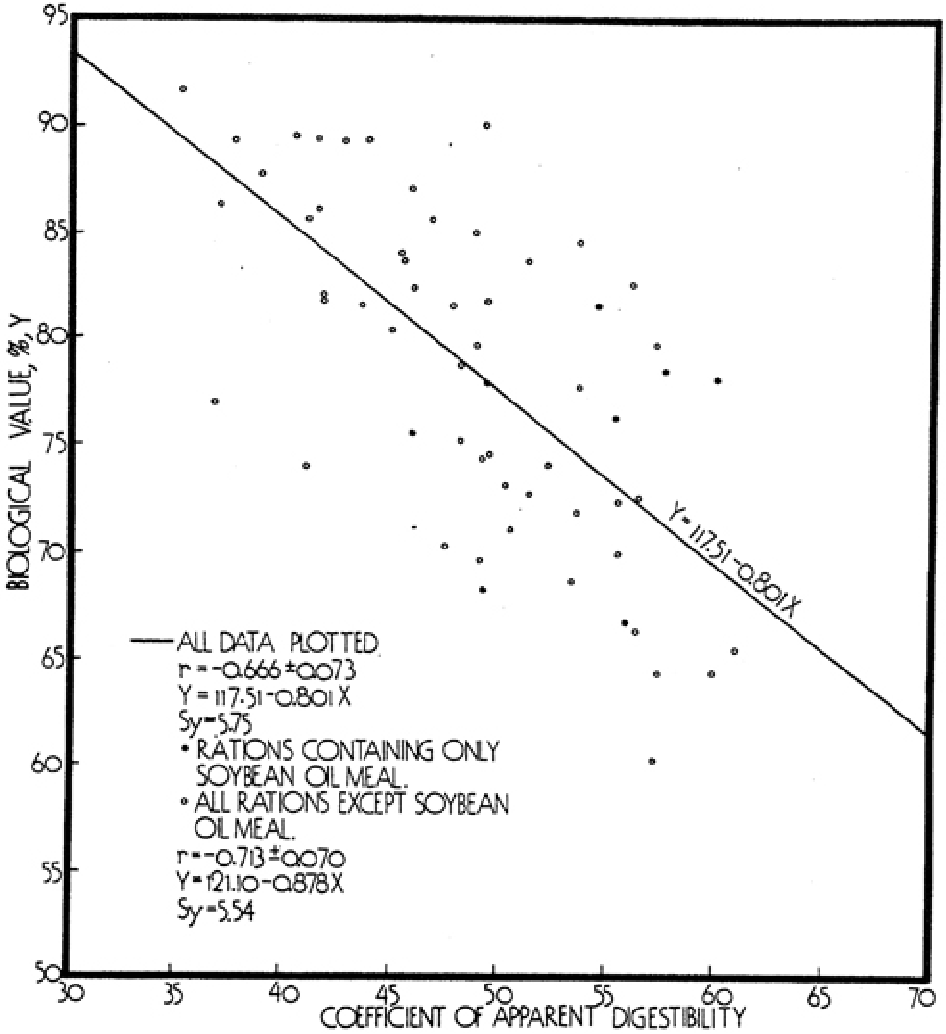


Fig. 11.—Biological values of protein in rations containing about ten per cent crude protein plotted against the coefficients of apparent digestibility of the crude protein of the rations. The equations, $Y = a + bx$, for the average regression lines are presented on the chart along with the coefficients of correlation and the standard errors of estimate.

body origin which may or may not be a result of the feed protein. The inverse relationship between the biological values and digestibility has already been brought to attention. This relationship is graphically presented in Fig. 11. The correlation coefficient when all of the 8.5 to 10 per cent protein rations were included in the analysis was —0.666± 0.073. When the soybean oil meal rations were omitted, the correlation coefficient was —0.713± 0.070. The evidence presented here thus indicates that for growing heifers fed rations containing similar amounts of protein and other nutrients, the efficiency with which the absorbed protein is utilized (biological value) is largely dependent upon how much of the protein is absorbed. The more that is absorbed, the lower is the biological value and vice versa. Morris (1938) and Ritzman and Benedict (1938) also observed an inverse relationship between percentage nitrogen utilization and the amount of nitrogen absorbed.

The Nutritive Ratio.—When the biological values were calculated for the all-hay rations which were used to determine the digestibility of lespedeza hay alone, about 20 per cent lower values were secured than when the hay was fed with starch and sugar. This difference seemed too great to be due only to the slight difference in concentration of protein in the ration. Hence, the possibility of a correlation between the biological values and the ratio of digestible protein

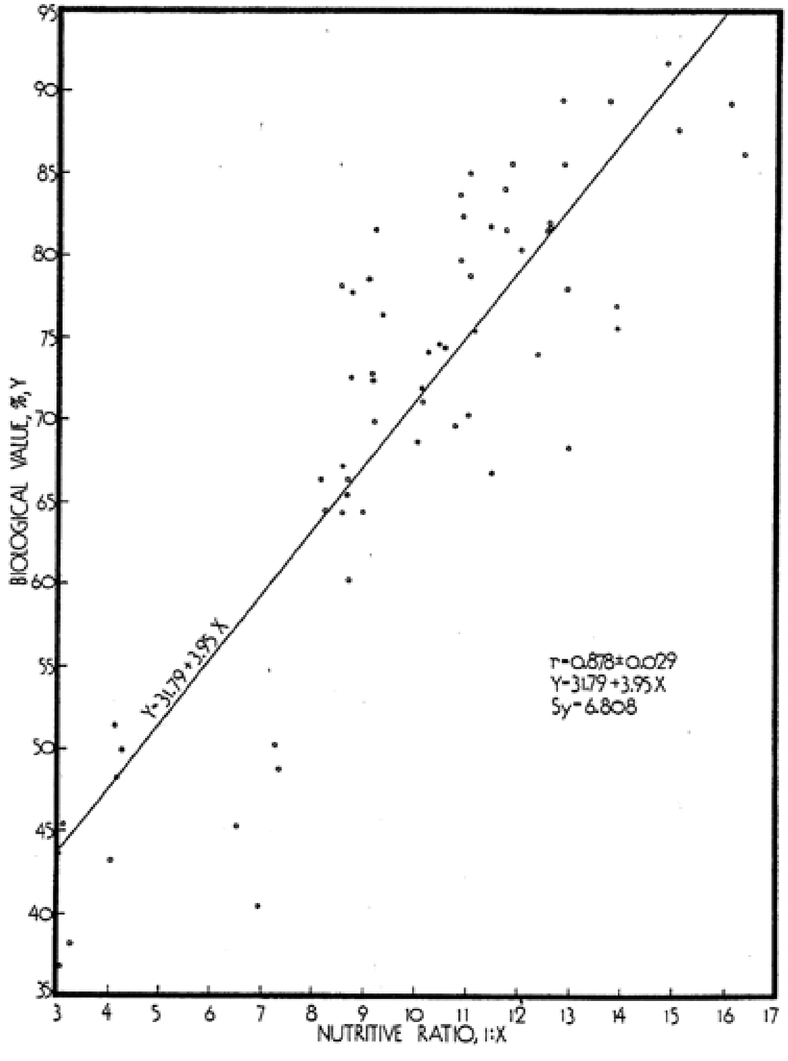


Fig. 12.—Biological values of protein in rations varying in content of total digestible nutrients and crude protein plotted against the nutritive ratios. The equation for the average regression line, the coefficient of correlation, and the standard error of estimate are printed on the chart.

to other digestible nutrients was investigated. Since such a correlation would be expected to hold for a wide range of nutritive ratios if it existed, all of the rations fed during the last two trials were included in the analysis. The date were ploted in Fig. 12. The correlation coefficient for biological values and nutritive ratios was 0.878± 0.029. There was a very pronounced tendency, therefore, for the biological values to increase as the nutritive ratio was widened.

This close relationship between biological values and nutritive ratios may logically be used as the basis for predicting the utilizable nitrogen in any ration for growing cattle for which the digestibility coefficients are known. The biological value of the protein may be obtained by the average equation $Y = 31.79 + 3.95X$, in which Y is the biological value and X is the second term of the nutritive ratio.

The data from ration 23 indicate that predictions made in the manner outlined above may give results that are too high if the total nutrient intake is too low to permit growth. All of the points for that ration were considerably below the average line of Fig. 12. The effect of feeding an excessive amount of feed may cause deviations from the average equation derived from these experiments, also. In any case, however, it is evident that predictions of utilizable nitrogen for growing cattle would be more accurate by this method than by the application of average biological values as found in the literature.

Importance of Biological Values for Growing Cattle

The evidence furnished by the experiments just reported indicates that biological values of feed proteins do not have the same significance for cattle as they do for rats and other non-ruminants. It would have been expected on the basis of rat experiments that milk protein would give the highest biological value in these experiments. Actually, it gave the lowest. This does not mean, however, that the milk protein had the poorest assortment of amino acids. Numerous trials with other animal species have ably established the superiority of milk proteins as compared to proteins of plant origin. As has been emphasized previously, the utilization of protein was not significantly different for most of the rations. Hence, since milk protein was absorbed the best, its percentage utilization necessarily had to be the lowest. In view of this inverse relationship between amount of protein absorbed and its biological value, it seems more important in ruminant nutrition to know the absorption, or digestibility, of the protein than its biological value. It has also been shown that the utilization of protein very definitely depends upon the ratio of digested protein to other digested nutrients; so the biological value of the protein is secondary to the digestibility of all of the nutrients and of their proportions to each other.

These relationships that have been shown for a wide variety of proteins fed at the same level of intake and a few fed at different levels are arranged very much as if the source of absorbed protein had been the same in all rations. Thus, lespedeza hay alone with an average nutritive ratio of 1: 8.51 gave a biological value of 66.98 and milk protein with an average nutritive ratio of 1: 8.76 gave biological value of 69.33 for the same heifers. When lespedeza hay was fed in a ration in which the nutritive ratio was 1:12.19 the biological value was 84.24. Milk and lespedeza in a ration with a nutritive ratio of 1: 11.47 gave a biological value of 83.50. If the absorbed nitrogen from the milk protein had actually been of superior quality for the heifers, larger differences should have been secured. The most logical explanation of the unvarying relationships observed between absorbed nitrogen, regardless of its source, and biological value is that the *digested proteins* from all of the rations were of nearly the same composition. The process by which they became of fairly constant composition is believed to be due to the activity of the microorganisms of the rumen in changing the character of the feed protein so that a large part of it is organismal in form before it is digested.

If these assumptions are correct, biological values secured with growing cattle have very little importance as far as the *feed protein* is concerned because *the proteins digested will not be the same as those fed.* The biological values relate only to the absorbed nitrogen, which will be derived from proteins that are quite different from those of the feed. Biological values applicable to the feed proteins could be secured only if these proteins were not used by the rumen microorganisms or not mixed with rumen microorganisms, and these conditions could be accomplished only in an animal whose rumen was free of microorganisms.

The biological values secured with growing cattle, do, nevertheless, have a definite biological significance. They indicated with the heifers of this experiment, just as they had in other animals, the amount of protein which the animal could utilize on any ration when the nutritive ratio and protein and feed intake were known. *The importance of the biological values of feeds for cattle, therefore, is more a matter of insuring the correct amount or proportion of digestible protein than of determining such very small differences in protein quality as may exist by the time the protein is ready for digestion.*

Digestibility Studies

When it became apparent that the efficiency of protein utilization was correlated with the amount of protein available and its proportion to other available nutrients seemingly irrespective of the feed source of the protein, investigations of the digestibility of the rations were instituted. In addition, the digestion coefficients of the various nutrients in lespedeza hay and lespedeza seed were determined.

Average Digestion Coefficients.—The feces of each heifer from each ration was analyzed during the last two trials and the coefficients of apparent digestibility were determined. These data were not all included here because of their bulk. The average coefficients are presented in Table 13 along with the nutritive ratios, the percentage of total digestible nutrients in each ration, and the average amount of the latter furnished to the animals each day. This table shows that the twelve rations used for determining biological values at about a 10 per cent level of protein all furnished similar amounts of total digestible nutrients. The heifers received from each of these rations, therefore, about the same amount of food for energy. The digestible protein supplied by the various rations was quite variable, however. The effect of this situation upon the utilization of the protein has already been discussed.

The role of lespedeza hay in causing the variations of digestible protein of the different rations is easily seen from an inspection of Table 13. The percentage of digestible protein furnished by les-

TABLE 13

AVERAGE DIGESTION COEFFICIENTS, NUTRITIVE RATIOS, AND TOTAL DIGESTIBLE NUTRIENTS OF RATIONS FED IN THE LAST TWO TRIALS

Ration		Coefficients of Apparent Digestibility							
No.	Description	Dry Matter	Crude Protein	Crude Fiber	Ether Extract	Nitrogen-free Extract	Total Digestible Nutrients	Nutritive Ratio	Total Digestible Nutrients Supplied Daily
		%	%	%	%	%	%	1:	lbs.
7	Lespedeza hay	60.99	39.25	40.34	34.16	77.37	56.08	15.2	8.97
8	Lespedeza hay	60.97	43.77	49.76	31.67	75.06	57.01	12.1	8.55
9	Alfalfa hay	63.72	50.30	31.87	14.11	83.45	57.37	10.6	8.61
10	Lespedeza and milk	64.43	48.08	35.33	35.41	81.51	60.23	11.5	9.04
11	Straw and milk	61.33	56.23	41.39	37.74	74.77	55.53	8.8	8.33
12	Lespedeza seed	59.15	51.66	50.24	39.05	72.94	56.19	9.8	8.43
16	Lespedeza hay	60.13	41.11	44.70	7.25	75.87	54.56	13.0	8.18
14	Soybean oil meal	64.05	56.85	53.11	48.65	75.13	57.38	9.0	8.61
17	Lespedeza and milk	63.66	47.04	30.30	2.59	79.37	56.79	11.1	8.52
19	Straw and milk	64.30	57.43	46.09	42.69	75.52	56.80	8.8	8.52
18	Lespedeza hay and soybean oil meal	61.90	43.94	32.02	17.61	79.11	54.63	11.9	8.20
15	Soybean oil meal	63.14	50.05	47.87	66.70	75.50	57.46	12.8	8.62
20	Lespedeza hay and seed	56.64	63.51	50.41	41.49	69.26	54.30	4.1	8.69
21	Lespedeza hay and seed	50.29	61.70	47.57	24.59	53.99	45.06	3.1	6.76
22	Lespedeza hay	57.95	49.17	54.21	29.15	68.90	52.03	8.5	8.33
23	Lespedeza hay	43.44	41.03	49.92	10.18	46.03	37.93	7.0	6.07

pedeza hay alone is the lowest of any of the rations to which starch and sugar were added to adjust the protein level. Furthermore, all rations which contained lespedeza hay were relatively low in digestible protein. Another noticeable change in the digestion coefficients when lespedeza hay was fed with another source of protein was a depression of the digestibility of crude fiber. Thus, while the digestibility of crude fiber of lespedeza hay fed with starch and sugar was about 45 per cent, when dried skimmilk was included in the ration it dropped more than 10 per cent. Soybean oil meal additions to lespedeza gave a similar effect. A possible explanation of this finding might be that the crude protein of lespedeza depends largely upon bacterial action in the rumen to free it. These rumen organisms in turn must secure their nitrogen supply from the hay; so they attack the hay in quest of nutrients. However, when there is at hand an abundance of easily secured nitrogen from milk or oil meal, the rumen organisms may use it in preference to attacking the hay.

The Effect of Lignin on Digestibility.—The suspicion of some factor preventing easy digestion of lespedeza hay protein prompted an investigation of its lignin content. Crampton and co-workers (1938, 1939 and 1940) had shown that by partitioning the carbohydrate portion of feeds into lignin, cellulose, and other carbohydrates they secured a more accurate idea of the value of the ration than from the conventional crude fiber and nitrogen-free extract partition. Furthermore, a small increase in lignin content of pasture herbage was shown to have a tremendous effect upon the availability of nutrients. Hale, Duncan and Huffman (1940) found that alfalfa containing 17.7 per cent lignin was much more poorly digested than hay containing 15.7 per cent lignin although the remaining composition of the two hays was practically the same. Hence, if lespedeza should be shown to be high in lignin, it might be an explanation of the poor digestibility of its protein and crude fiber.

Data showing the comparison of the lignin content of the lespedeza hays used in this experiment with the lignin of the alfalfa hays are presented in Table 2. The lespedeza hay, even that highest in protein, contained more than 50 per cent more lignin than the alfalfa hay. Lignin comprised nearly one-fifth of the total dry matter of the lespedeza hays. Such high amounts of lignin are usually found only in ripened grasses (Patton, 1943), which have often been shown to be of poor nutritive value.

It was observed that, with the exception of the very late-cut lespedeza hay, the more leafy the hay, the higher the lignin content. A comparison of the composition of lespedeza leaves and stems was therefore made. For this purpose a bale was selected from each of three different lots of Korean lespedeza hay. The leaves were stripped from the stems by hand and the parts carefully separated. Two of the bales were intermediate-cut hay and the third was late-cut hay. The results of the analyses are given in Table 14 along with corresponding figures for alfalfa leaves and stems secured in a similar manner. The data in Table 14 show that the lespedeza leaves were highly lignified when the plant was cut for hay while alfalfa leaves at the hay making stage were only slightly lignified. Otherwise, lespedeza and alfalfa leaves were similar in composition, and lespedeza and alfalfa stems were quite similar in composition.

TABLE 14

COMPOSITION OF THE DRY MATTER OF LESPEDEZA AND ALFALFA LEAVES AND STEMS

Sample	Portion	Lignin	Cellulose	Other Carbohydrates	Crude Protein	Ether Extract	Mineral Matter	Crude Fiber	Nitrogen-free Extract
		%	%	%	%	%	%	%	%
Lespedeza_1	Stems	17.52	34.54	33.28	9.09	2.07	3.50	39.12	46.23
Lespedeza_2	Stems	16.68	38.49	33.84	6.70	1.13	3.15	48.64	40.37
Late lespedeza_3	Stems	18.60	37.62	31.82	7.02	1.48	3.46	48.15	39.89
Lespedeza_1	Leaves	24.59	23.21	19.31	18.13	4.71	10.05	19.11	48.00
Lespedeza_2	Leaves	23.67	26.45	22.85	17.80	3.48	5.76	22.81	50.16
Late lespedeza_3	Leaves	22.03	25.86	20.10	21.36	4.15	6.51	24.14	43.85
Ave. lespedeza	Stems	17.60	36.88	32.98	7.60	1.56	3.37	45.30	42.16
Ave. lespedeza	Leaves	23.43	25.17	20.75	19.10	4.11	7.44	22.02	47.34
Alfalfa	Stems	15.61	43.21	24.19	8.93	1.12	6.94	50.45	32.56
Alfalfa	Leaves	8.23	20.74	34.10	24.07	2.96	9.90	16.62	46.46

It seems reasonable to assume that the low digestibility of lespedeza hay protein is quite probably due to the very high lignin content of the part of the plant bearing most of the protein, the leaves. This theory receives added weight from the experiments on the bacterial decomposition of organic matter by Waksman and Iyer (1933) in which they found that lignin and proteins form complexes which are quite resistant to attack by microorganisms. The importance of rumen microorganisms in ruminant digestive processes makes an analogy quite logical. The lespedeza protein may be bound to or protected by lignin so that it is not released or utilized by the rumen microorganisms as readily as proteins of less lignified roughage or concentrate feeds. Hale, Duncan and Huffman (1940) found that lignin was not digested appreciably in the rumen; so the rumen microorganisms probably can not effectively attack highly lignified material.

Indirect evidence that rumen fermentations may account for some lignin digestion was obtained by comparing digestion coefficients of rations which were mainly lespedeza hay with those of lespedeza hay supplemented by lespedeza seed or milk. These comparisons are presented in Table 15. It is shown there that addition of milk to intermediate-cut hay reduced the digestibility of the lignin, cellulose and crude fiber. The same effect occurred when lespedeza seed was added to a ration of intermediate-cut hay. Lespedeza seed in a ration with oat straw also resulted in no digestion of lignin and poor cellulose digestion. The digestibility of the fibrous portions of very highly lignified late-cut lespedeza hay was not greatly affected by lespedeza seed supplementation, however, as shown by comparing rations 23 and 21.

TABLE 15

THE EFFECT OF PROTEIN SUPPLEMENT UPON THE DIGESTIBILITY OF LIGNIN, CELLULOSE AND CRUDE FIBER OF LESPEDEZA HAY AND SEED

Ration No.	Description	Coefficients of Apparent Digestibility Lignin	Cellulose	Crude Fiber	Other Carbohydrates
		%	%	%	%
7	Lespedeza and sugar	11.08	55.90	40.34	89.17
8	Lespedeza, sugar, Starch	20.25	56.44	49.76	90.97
10	Lespedeza and milk	0.00	49.24	35.33	93.07
22	Lespedeza hay alone	17.99	63.18	54.21	84.91
20	Lespedeza hay and seed	6.62	58.69	50.41	88.76
23	Lespedeza hay alone	10.60	56.34	49.92	74.01
21	Lespedeza hay and seed	14.80	54.58	47.57	78.37
12	Oat straw and lespedeza seed	0.00	49.45	50.24	87.77

The apparently depressing effect of milk and lespedeza seed upon digestibility of lespedeza hay may have been due to a preferential use of the concentrated feeds by the rumen flora (as previously mentioned) or to some change of the types of rumen microorganisms effected by the feeds. The latter probability has been suggested by Mills, Booth, Bohstedt and Hart (1942) from their study of urea utilization by the rumen flora in the presence of casein. If such is the case, the microorganisms favored by the concentrated feeds may be poor utilizers of highly lignified feeds such as lespedeza. Further evidence of the depression of digestibility in highly lignified feeds is presented below in a comparison of digestion coefficients of intermediate and late-cut hay.

Digestibility of Lespedeza Hay and Lespedeza Seed.—Digestion coefficients of intermediate-cut hay were determined with the four heifers of the second group. Late-cut hay of high lignin content was used in determining digestion coefficients with the third group of heifers. Ground lespedeza seed was fed with each hay at the rate of one part of seed to three parts of hay in order to determine the digestion coefficients of the seed. The calculated digestion coefficients for each heifer and the averages are presented in Table 16. The compositions of the hays and seed were given in Table 2 and Table 3.

TABLE 16

DIGESTION COEFFICIENTS OF LESPEDEZA HAY AND LESPEDEZA SEED

Ration	Heifer No.	Coefficients of Apparent Digestibility								Total Digestible Nutrients
		Dry Matter	Crude Protein	Crude Fiber	Ether Extract	Nitrogen-free Extract	Cellulose	Lignin	Other Carbohydrate	
		%	%	%	%	%	%	%	%	%
22 Intermediate lespedeza hay	24	56.43	48.27	55.71	25.79	66.67	63.15	17.16	83.61	51.22
	26	57.67	50.57	56.06	25.99	68.13	64.54	14.35	86.14	52.20
	27	58.73	51.95	53.75	29.44	70.21	63.56	19.34	85.33	52.78
	28	58.95	45.89	51.32	35.37	70.57	61.48	21.10	84.57	51.94
	Average	57.95	49.17	54.21	29.15	68.90	63.18	17.99	84.91	52.03
23 Late lespedeza hay	46	43.66	41.93	48.83	17.11	47.26	56.32	10.61	74.52	38.49
	47	42.35	39.49	51.15	13.58	44.42	57.06	12.36	70.15	37.75
	48	45.05	40.38	52.43	6.40	47.02	57.52	13.30	75.47	38.82
	49	42.71	42.32	47.27	3.64	45.43	54.47	6.14	75.89	36.65
	Average	43.44	41.03	49.92	10.18	46.03	56.34	10.60	74.01	37.93
20 Intermediate hay and seed	24	56.61	63.63	53.82	41.46	70.19	61.45	0.94	93.05	55.50
	26	58.63	65.58	55.02	47.82	69.04	62.24	5.74	89.74	56.28
	27	56.08	61.93	46.16	34.42	68.31	55.63	8.42	85.64	51.95
	28	55.24	62.90	46.62	42.24	69.48	55.43	11.39	86.60	53.46
	Average	56.64	63.51	50.41	41.49	69.26	58.69	6.62	88.76	54.30
21 Late hay and seed	46	51.96	63.39	48.94	24.51	55.01	55.07	12.86	83.67	46.10
	47	51.45	62.79	49.49	29.30	54.41	56.38	17.00	78.04	46.26
	48	50.41	59.50	50.38	19.99	54.35	56.92	18.31	77.34	45.25
	49	47.34	61.11	41.45	24.54	52.19	49.95	11.03	76.43	42.62
	Average	50.29	61.70	47.57	24.59	53.99	54.58	14.80	78.87	45.06
Lespedeza seed (by difference)	24	57.15	78.79	35.68	55.90	82.92	45.96	0	100.00	
	26	61.39	80.39	44.58	67.93	72.34	46.72	0	100.00	
	27	48.40	71.76	0	39.01	61.43	2.20	0	86.62	
	28	44.50	79.69	3.02	48.56	65.54	14.69	0	93.13	
	46	75.86	84.20	49.78	34.94	81.60	47.24	13.72	100.00	
	47	77.64	85.38	36.70	51.43	88.68	52.14	56.74	100.00	
	48	65.84	78.06	34.63	39.12	79.52	53.15	40.88	82.56	
	49	60.69	79.32	0	53.96	75.37	21.54	56.25	77.94	
	Average	61.43	79.70	25.55	48.86	75.93	35.46	20.95	92.53	62.40

The average digestion coefficients of the intermediate-cut hay compare favorably with similar coefficients for clover hay published by Morrison (1936) *except for the coefficient of apparent digestibility of crude protein.* The average of 49.17 per cent secured from lespedeza is 10 per cent or more below similar coefficients published for clover hay. The effect of this is a widening of the nutritive ratio of lespedeza hay from a previously estimated 1:4 or 5 to an actual ratio of 1: 8.5. As far as protein nutrition is concerned, this puts lespedeza hay in the same class as mixed legume and non-legume roughages.

The tremendous effect of maturity and increased lignification of lespedeza hay upon its digestibility is shown by comparing the average results for late and intermediate-cut hay given in Table 16. The digestibility of every nutrient of the late-cut hay was markedly depressed, with the greatest decreases occurring for protein, ether extract, and nitrogen-free extract. The late-cut hay contained some immature seed which was not crushed by the hammer mill. This seed was apparent in the feces but it was such a small portion of the total that its contribution to the decreased digestibility was considered very slight. It is believed that the high degree of lignification of the late-cut hay (23.14 per cent of its dry matter was lignin) was primarily responsible for its generally poor digestibility.

The digestion coefficients of Korean lespedeza seed determined by difference are presented at the bottom of Table 16. There was considerable variation in the coefficients so determined for some of the nutrients, notably crude fiber, cellulose and lignin. Since these make up such a small amount of the seed, however, the variations do not seriously affect the estimation of the nutritive value of the seed. The values secured with all eight heifers were averaged together because of the close similarity in composition of the seed fed to each group, and, except for lignin, the apparent absence of effect of the late-cut hay upon digestibility of the seed. The digestion coefficients, except for crude fiber, are comparable to those published by Morrison (1936) for cottonseed meal and other high protein concentrates of protein content similar to that of lespedeza seed. The crude fiber of lespedeza seed is not exceedingly high, but it is mostly from the very tough hulls which adhere to the seed and are quite high in lignin.

The average total digestible nutrients of lespedeza seed given in Table 16, 62.4 per cent, was calculated on the basis of the composition of the seed fed in ration 21. The average percentage of digestible protein of that seed was 28.98, which gave a nutritive ratio of 1:1.15. Ground Korean lespedeza seed should, therefore, be a valuable high protein concentrate. This conclusion has been confirmed by Herman and Ragsdale (1942) who found it very satisfactory in rations for milking cows.

DISCUSSION

This investigation has proceeded with two purposes; first, the evaluation of the nutritive value of the crude proteins of lespedeza hay and seed; and, second, an analysis of the importance of biological values of proteins fed to growing cattle. After further discussion of the latter point, a basis for evaluating feed proteins for dairy cattle will be provided; and the proteins of lespedeza hay and seed will be discussed on that basis.

A discussion of the importance of biological values secured with cattle has already been presented. It was shown that their application to the proteins of the feed is probably erroneous because the microorganisms of the rumen may alter a large part of the feed protein by their activity. This theory has also been presented by Johnson, Hamilton, Mitchell and Robinson (1942) in connection with their study of nitrogen utilization by sheep. The validity of it is further emphasized by the investigations of Wegner, Booth, Bohstedt, and Hart (1940, 1941 and 1941a) and Mills, Booth, Bohstedt and Hart (1942) concerning the utilization of urea by cows and calves. They showed that rumen microorganisms rapidly converted urea nitrogen to ammonia and then utilized the ammonia in the synthesis of their body protein. When the rations did not contain readily soluble carbohydrate, urea was poorly utilized because of the inadequate growth of the rumen microorganisms. The microorganisms also seemed to prefer protein to the ammonia from urea because when the ration was high in protein the urea was transformed to ammonia, but the ammonia was not readily synthesized into bacterial protein. There is, therefore, considerable evidence to indicate that the ruman flora grow at the expense of the feed and that they may construct a large part of the feed nitrogen or protein into microorganismal protein.

The question whether or not the protein so constructed by the rumen flora could be utilized by the animal has recently been definitely answered in the affirmative. Fingerling and co-workers (1937) proved by nitrogen balance experiments with steers that urea nitrogen was utilized for growth. Bartlett and Cotton (1938) and Hart, Bohstedt, Deobald and Wegner (1939) demonstrated the same fact by long time feeding experiments with calves. Harris and Mitchell (1941 and 1941a) have also presented critical evidence of efficient use of urea nitrogen for growth by sheep. The use of the urea nitrogen has been shown by Wegner et al. (1941) to be by way of bacterial protein developed in the rumen.

The relative value of the feed protein and the microorganismal protein as sources of essential amino acids is still an unsettled question. Smuts, Du Toit and van der Wath (1941) concluded that the rumen flora could not synthesize cystine and that their action on the proteins of the feed was "purely a question of interception."

Hamilton (1942) found, however, that when the rumen flora were furnished a source of inorganic sulfur, expected cystine supplementation did not occur. The proper nutrition of the rumen flora may therefore be an important factor in the most efficient utilization of feeds. The high biological value of microorganisms is suggested from experiments with yeast protein. Mitchell (1942b) found that yeast protein was utilized by rats nearly as efficiently as milk protein. Schundt, Schleinitz, and Lagnean (1934) in experiments with pigs found that yeast protein was used more efficiently than either peanut or soybean protein, which are both considered good quality proteins.

Because of the activity of the rumen flora, the impossibility of correlating biological values of feed proteins secured with rats and ruminants is thus apparent. It has been shown, furthermore, that the biological values secured with growing heifers were primarily dependent upon the nutritive ratio in an adequate diet. In other words the digestibility of protein and of the non-nitrogenous nutrients are the most important factors, if not the only ones, practically necessary to consider in evaluating the nutritive value of feed proteins. If further confirmation is desired, the results of nitrogen balances (preferably long-time balances to reduce variation) will give data from which biological values and other desired indexes of nutritive value may be computed by use of the average formulae for determining endogenous urinary and fecal metabolic nitrogen. The use of the biological values of proteins as a comparison of their over-all nutritive value must not be made unless the nutritive ratios as actually determined are the same. Other methods of expressing the nutritive value of protein are more closely correlated with actual nitrogen storage and utilization. The application of these various methods has been discussed. When the results of this investigation are compared in the light of these developments with similar investigations made with sheep, substantial agreement is achieved. The reason more variable biological values were not secured by Miller and Morrison (1942) is undoubtedly the similarity of the nutritive ratios of the rations they fed.

Clearly, the question of the nutritive value of the protein of lespedeza hay or seed must be answered with qualifications. This investigation has revealed that for dairy heifers the quality of protein resulting from lespedeza hay or seed is equal to that from milk, alfalfa hay, corn, and probably soybean oil meal if proper allowance is made for the different nutritive ratios of the rations. If it will be accepted that milk protein will result in a high quality of digestible protein for heifers, then the quality of protein resulting from lespedeza hay or seed for dairy heifers must unquestionably be high. The relative quantity which will be digested is another matter.

The digestibility studies showed that the crude protein of ground lespedeza seed was quite digestible and comparable with other high protein supplements of the same nitrogen content. The protein of ground lespedeza seed must be accepted as being of high nutritive value for dairy heifers, therefore, being both readily absorbable and of good quality. The actual efficiency with which it will be utilized will depend upon the type of ration in which it is fed, such as the nutritive ratio and amount of feed furnished in relation to the animal's requirements.

The crude protein of lespedeza hay has been shown to be relatively poorly digested, resulting in a smaller amount of absorbed nitrogen from lespedeza hay than from other hays of similar nitrogen content. This smaller supply apparently did not adversely affect the utilization of the protein by the heifers in these experiments. However, if conditions were such that the amount of protein was the limiting factor of the value of any ration, the total protein of lespedeza hay would be less valuable than that of any of the feeds which were investigated simply because it would result in a smaller percentage of utilizable nitrogen. In ordinary practice where protein is often used wastefully, the feeding of lespedeza hay according to generally used standards should not result in a protein crisis. The poor digestibility of the protein of lespedeza hay must be considered a potential deficiency in evaluating its nutritive value, however.

Although the highly lignified leaves of lespedeza hay have been proposed as an explanation of the low digestibility of its protein, there may be other important factors concerned. The results from the late-cut hay leave little doubt, however, that lignification or something associated with it as the plant matures results in a lessened digestibility of nutrients, especially protein. The results of the digestion trials with late-cut hay clearly demonstrate the importance of cutting Korean lespedeza hay at an early stage. The intermediate-cut hay averaged 37 per cent higher in total digestible nutrients than the late-cut hay. This is strong indication that an acre of hay cut before bloom (as the intermediate-cut hay) would yield a much greater quantity of digestible nutrients than an acre cut after the bloom stage.

SUMMARY

An investigation has been made of the utilization of the crude protein (N x 6.25) of Korean lespedeza hay and seed and various other feeds by growing dairy heifers. For this purpose methods were developed for the application of the nitrogen balance method of determining the biological values of feed proteins to use with yearling dairy heifers.

The average excretion of fecal nitrogen (fecal metabolic nitrogen) on a low nitrogen ration was 5.3 grams per kilogram of dry feed consumed. Endogenous urinary nitrogen was found to vary as the

0.42 power of body weight and a formula was developed to estimate the endogenous urinary nitrogen for heifers of various weights.

Biological values of the various proteins were determined, and net protein values and other measures of the nutritive value of the proteins were calculated. The net utilization of proteins from lespedeza hay, alfalfa hay, dried skimmilk, corn, lespedeza seed, soybean oil meal, and combinations of lespedeza hay with corn, milk, or soybean oil meal was not significantly different for dairy heifers when they were fed at a 10 per cent level. The feeds were ranked according to the biological value of their proteins as follows: lespedeza hay, corn and lespedeza hay, milk and lespedeza hay, alfalfa hay, corn, soybean oil meal, soybean oil meal and lespedeza hay, lespedeza seed, and dried skimmilk. The same feeds and combinations ranked according to digestibility approximately the reverse of their order for biological values.

Significant supplementary actions between the proteins of lespedeza hay and corn, soybean oil meal, or milk were not found.

Important correlations between the biological values and the amount of nitrogen absorbed, the true digestibility, and the apparent digestibility of the crude protein were demonstrated for rations which contained similar amounts of protein and total digestible nutrients. A high correlation, $r = 0.878 \pm 0.029$, was found between biological values and the nutritive ratios of a wide variety of rations. When different proteins were fed at the same nutritive ratio they gave similar biological values.

The importance of the various methods of expressing the nutritive value of feed proteins for growing dairy heifers, and possibly other ruminants, has been discussed. The very active role of the rumen microorganisms in the protein nutrition of ruminants has been reviewed as an explanation of the slight differences observed between the net utilization of a wide variety of proteins. It was concluded that the quality of the absorbed proteins from Korean lespedeza hay or seed was equal to the quality of the absorbed proteins from milk, corn, alfalfa hay, or soybean oil meal for growing dairy heifers. The error of using biological values to express the nutritive value of feed proteins for ruminants was emphasized.

The digestibility of the crude protein of lespedeza hay was shown to be relatively low, and the high lignin content of lespedeza leaves was revealed as a possible explanation of the poor protein digestibility. Highly lignified late-cut lespedeza hay was shown to be of very low nutritive value, the digestibility of all of its nutrients being greatly depressed. Digestion coefficients were determined for all of the nutrients of intermediate-cut lespedeza hay, late-cut lespedeza hay, and ground lespedeza seed.

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