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FACTORS AFFECTING THE BIOLOGICAL VALUE

OF OAT PROTEINS

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TABLE OF CONTENTS

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INTRODUCTION	•	•	0	•	•	•	•	•	•	•	•	1
LITERATURE REVIEW	•	•	•	•	•	•	•	•	•		•	2
EXPERIMENTAL PROCEDURE		•	•	•	•	•	•	•		•	•	7
RESULTS AND DISCUSSION	•	•	•	•	•	•		•	•	•	•	16
SUMMARY	•	•	•		•	•	•		•	•	•	32
BIBLIOGRAPHY												34

INTRODUCTION

Since 85 per cent of the cat crop is used for the feeding of livestock, the composition of this grain, particularly the biological value of the protein is an important factor in animal nutrition. As has been the case with other cereal grains, plant breeding has developed new high-yielding, disease-resistant varieties of cats with increased protein content. From the agronomic standpoint, this work has been fruitful in the production of new varieties which have proved satisfactory in regard to yield, disease and insect resistance and protein content. Comparatively little is known, however, of the nutritive value of these varieties.

The purpose of the present study was to determine the effects of variety, date of planting, and location of planting on the biological value of the protein of several varieties of oats grown in Oklahoma. Experiments were also carried out to determine the effect on rat growth of the addition of two amino acids, lysine and methionine, to a diet in which oats were the sole source of protein.

LITERATURE REVIEW

Investigators agree that none of the common cereal grains, when fed alone, afford satisfactory nutrition (5, 6, 19, 17, 24, 26). Osborne and Mendel (27) showed that this is due to a deficiency in certain of the dietary components, notably mineral salts and fat soluble vitamins, and to the fact that some of the grain proteins are biologically incomplete.

Although there are contradictions in the early literature in regard to the nutritive value of oat protein, the general conclusions are that oat protein has a somewhat higher biological value than that of other cereals, rye, corn, wheat and barley (17, 27, 18). Hoagland and Snider (14) noted the close similarity between the protein of oat meal and wheat, while Hartwell (10) found that the protein of oats was of comparatively good nutritive value if supplemented only with butter and mineral salts.

Prior to a complete knowledge of the vitamins and essential amino acids required for growth, Mc Collum and his associates (20), following numerous observations regarding the nutritive value of the oat kernel, stated:

We have not yet been able to supplement oats with purified food ingredients and attain optimum results, when the oat kernel constituted from 70 to 80 per cent of the food mixture. Gelatin combined with oat proteins forms a much better protein mixture than do casein and oat protein. . . . We have not yet determined the cause, but it is evident that a high intake of oats over a long period causes injury to the rat.

Osborne and Mendel (27) were able to obtain considerable growth on oat diets containing as low as five per cent protein, and they were of the opinion that their results conflicted with those reported by McCollum because of their use of starch rather than dextrin and agar in the ration. This will not, however, serve to explain the differences found. In view of more recent investigations (8), if the differences observed were due to intestinal-flora synthesis of the B-complex vitamins, it would appear that dextrin would be better than starch for the synthesis of growthpromoting factors. It was further pointed out by Osborne and Mendel (27) that the main confusion existing in the literature regarding the nutritive value of oats may be attributed to the conflicting statements made even by the same author. For example, in one report Mc Collum (21) stated: "The oat kernel seems to contain proteins of a poorer quality than either maize or the wheat kernel." In another publication (22) he reported:

The protein of the cat kernel has a slightly higher value for growth than has that of either wheat or corn, but the amount furnished by 90 per cent of rolled cats is below the optimum for the support of growth in a rapidly growing species.

Following the elucidation by Rose (3) of the nutritive significance of the essential amino acids and related compounds, investigations have been made which indicate that the nutritive value of oats is superior to that of other cereal grains. Mitchell and Smuts (25) determined the effect of lysine supplementation on the growth-promoting value of a diet in which whole oats were fed to provide protein at an eighth per cent level. Over a nine-week period the average gain in weight was 1.38 grams per day for the rats which received lysine, as compared to 1.30 grams per day for the rats receiving the unsupplemented ration. The authors stated that it is reasonable to expect a more marked difference in growth as a result of the addition to the diet of an amino acid in which the protein is most deficient, and they were of the opinion that a secondary amino acid deficiency may have developed as a result of the addition to the diet of minute amounts of the amino acid in which the protein was primarily

deficient, and that this might explain the slight difference they observed in the growth-promoting values of the supplemented and unsupplemented rations. The authors concluded that the proteins of oats and wheat are deficient in lysine, a fact confirmed by other workers (5), and that in the case of oats, addition of lysine resulted in a distinct, but small, increase in the growth-promoting effect.

The relative amino acid deficiencies in a protein can be determined by a comparison of the proportions of essential amino acids in the protein with the amounts of amino acid required for growth by rats, or some other organism. The work of Sherman and Woods (32) and that of Grau and Almquist (7) has demonstrated the usefulness of the rat assay method in studying the biological value of proteins. The latter authors used this method to determine the methionine content of various proteins in feeds.

Mitchell and Carman (24) were the first to show that for the rat, whole egg protein is an almost perfect protein from a biological standpoint; this fact was later confirmed by Sumner (33). Furthermore, it has been shown that casein, although a protein of high quality (2, 25), is slightly deficient in cystime and methionine relative to whole egg protein. Mitchell and Elock (23) found that the biologic value of casein is 73 per cent that of egg protein, and the biological value of the protein of oats is 66 per cent relative to egg protein. The authors assumed that the biological value of proteins was dependent entirely on their content of essential amino acids, and proposed that, until accurate values for the amino acid requirements of the growing rat are established, the best way to show the quality of a protein is to calculate the percentage deficit of each amino acid compared with the amino acid content of a protein mixture which is utilized completely by the rat. When the proteins were arranged in an order of increasing deficits, they were found to be roughly in order of decreasing nutritional value. Between the maximum deficit and the biological value, the correlation coefficient was calculated as r = -0.86, which was statistically highly significant. An approximate estimation of the biological value of a protein when its maximum deficit of essential amino acid is known was calculated from the regression equation relating the two variables Y (biological value) and X (maximumdeficit) in which: Y = 102 - 0.634 X. That this method does not always compare favorably with chemical means is pointed out by later investigators; however, the comparison of the chemical and biological methods clearly supports the hypothesis that the biological value of a protein is due to its content of essential amino acids.

Harte <u>et al</u>. (9) demonstrated the usefulness of the rat assay in determining the biological value of proteins. They concluded that randomly selected rats give precisely the same growth response as groups from the same stock which were paired with respect to sex and litter. It was also pointed out that partial restriction of food intake to not more than ten grams daily reduced the mean growth response by approximately 20 per cent from the mean growth of ad libitum fed animals and that only one-eighth to one-tenth as much variance was observed for the restricted-fed animals as for the ad libitum fed animals.

Osborne and Mendel (28) were the first to originate and define the term "protein efficiency" as the ratio between grams gain in weight and the amount of protein consumed. This term was proposed as a numerical expression of the nutritive value of proteins, as demonstrated by their growth-promoting ability in young rats. They presented evidence which indicated that variations in the level of dietary protein affected the

value obtained for protein efficiency; consequently it was suggested that the level of maximum protein efficiency should be determined for each protein if a true comparison of the biological value was to be found. With this knowledge at hand, Hegsted and Worchester (13) determined the protein efficiency for growing rats in a series of experiments, and notwithstanding the wide difference in the nutritive value of the proteins used, namely skim-milk powder, corn germ, yeast and peanut flour, the protein mixtures were classified in the same order with respect to each other regardless of whether protein efficiency or weight gain was used as a measure of nutritive value. The authors analyzed the covariance between gain in weight and protein efficiency, and were of the opinion that within the limits of their experiment there was a difference in the growth-promoting ability of proteins even after corrections were made in the covariance analysis to equalize food intake, and that the nutritive value of a protein could be measured by the use of gain ir weight alone.

More recently Heathcote (11), employing chiefly microbiological methods, determined the amounts of 18 amino acids appearing in oat protein and related per cent of the essential amino acids to protein quality. He compared his results with those of Block and Bolling (3), who compiled data derived from various sources for 12 amino acids appearing in oat proteins, determined chiefly by chemical methods. Heathcote concluded that there were no significant varietal differences in the amino-acid content of the oats tested. It was also pointed out that 85 per cent of the total nitrogen found in the oat kernel could be accounted for in terms of amino acid and amide-nitrogen.

EXPERIMENTAL PROCEDURE

Production of Oats

Grains used in this investigation were provided by Dr. A. M. Schlehuber of the Agronomy Department at Oklahoma Agricultural and Mechanical College. The cats were planted with a grain drill in field plots, and were harvested by hand to avoid mixing of the different varieties. Except in those cases in which the effect of date of planting was studied, winter cats were planted around September 21, the optimum planting date in the fall, and the spring varieties were planted on March 1. After harvest, the grains were threshed, cleaned and weighed. Upon delivery to the Agricultural Chemistry Research Laboratory the sacked grains were stored for a short time prior to the assay.

The following varieties of oats were grown and subsequently employed in this investigation: Wintok, Tennex, Forkedeer, Stanton Strain I, Traveler, Desoto, Andrew, Cherokee and Neosho. Of the oat varieties mentioned, all but the last three are winter varieties. Casein and one wheat variety, Comanche, were also used in each experiment to serve as controls; this variety of wheat has shown exceptional yield and protein content when grown in 24 tests in eastern Oklahoma and in many respects is the most desirable wheat grown in Oklahoma today (29).

Methods of Chemical Analysis

A sample of each protein source used in the diets was finely ground in a Wiley mill, and analyzed for the following constituents: Moisture, ash, ether-extract, total nitrogen, and crude fiber. The procedures employed were the conventional methods outlined in the A. O. A. C. Methods (1). Since these methods are generally well known, they need only brief description here.

<u>Moisture</u>: A two-gram sample was heated at 105° C for six hours, then weighed and the loss of weight determined.

<u>Ash</u>: Using the samples from the moisture determination, the residue was ashed in an electric muffle furnace maintained at 625° C for two hours. <u>Ether-extract</u>: Dry, two-gram samples were extracted in fat tubes with anhydrous diethyl ether for 16 hours and afterwards dried and reweighed; the difference between this and the initial dry weight was the weight of the ether-soluble material.

Total nitrogen by the Kjeldahl-Gunning procedure: Two-gram samples of grain or 0.5-gram samples of casein were analyzed for total nitrogen using this conventional method, with CuSO₄-Na₂SO₄ as a catalyst. The per cent nitrogen obtained was converted to per cent protein using the factor 6.25. All samples for nitrogen were run in triplicate.

<u>Crude fiber</u>: Residues from the ether extractions were digested with dilute H_2SO_4 and afterwards with dilute NaOH. They were filtered through linen after each digestion with the aid of suction. The remaining residue was dried, weighed and ashed, the loss in weight being the crude fiber.

Proximate composition of each protein source used in the experiments is given in TABLE I.

Table I

Composition of protein source.

Protein source	Moisture %	Ash %	Ether- extract	Total protein %	Crude fiber
		Experi	nent I	1	87.S.H.
Casein	9.13	1.75	0.37	89.84	
Wheat	11.83	1.78	1.63	13.41	2.17
Wintok	10.95	2.93	6.12	15.75	7.29
Cherokee	11.42	3.57	3.19	16.56	10.21
		Experi	ment II		
Casein	10.71		_	87.12	
Wheat	12.47	1.75	1.44	13.33	1.87
Wintok	10.17	2.72	5.95	15.61	9.01
Cherokee	7.37	3.52	2.78	15.84	11.13
		Experi	nent III		
Casein	12.63	_	_	86.06	-
Wheat	11.73	1.30	1.92	12.39	2.17
Wintok	9.96	3.25	4.29	13.46	8.73
Tennex	10.18	3.60	4.06	13.13	8.64
Forkedeer	10.44	3.48	4.69	12.59	9.83
Stanton Str. I	9.85	3.72	4.89	13.46	9.87
Traveler	9.36	3.40	4.67	12.85	9.50
Desoto	9.48	3.55	3.95	12.52	11.41
Andrew	11.26	3.29	3.44	12.62	7.54
		Experi	nent IV		
Casein	11.78	6× 274		83.60	-
Wheat	10.72	1.49	2.60	11.20	2.17
Neosho Date I	8.17	3.76	3.72	12.63	11.06
Neosho Date III	I 6.76	3.49	5.22	13.42	9.89
Andrew Date I	8.31	3.29	4.87	12.70	7.66
Andrew Date III	I 7.71	3.48	4.93	11.55	9.26
Forkedeer (Perl	c.) 8.65	3.92	5.31	12.75	10.75
Forkedeer (L. H	8.) 8.65	3.42	6.28	12.48	8.82
Tennex	8.35	3.30	7.19	14.72	7.21

* Oat varieties I planted March 1, 1951, and III planted March 20, 1951. ** Forkedeer was grown at two locations, Perkins and L. Blackwell, Okla.

Preparation and Composition of Rations

In each experiment, the level of protein in all rations was held constant and was adjusted by the addition of corn starch; and in all experiments except the first, fiber content of the rations was equalized by the addition of "solka-flock"¹, a pure cellulose material. A salt mixture (Hegsted, 12) was introduced at a four per cent level, and an adequate supply of the B-complex vitamins was assured by the addition of a vitamin mixture at the level per kilogram of ration shown in TABLE II.

TABLE II

Amount (mg.) Vitamin per kilogram of ration Thiamin 4.0 Riboflavin 6.0 Pyridoxine HCl 3.0 Nicotinic Acid 20.0 Ca Pantothenate 20.0 Inositol 20.0 Para amino benzoic acid 20.0 Pteroylglutamic acid 0.5 Choline ° Cl 100.0

Composition of vitamin mixture

In preparing the rations the weighed dry ingredients were placed in a feed mixer and after they were thoroughly mixed, corn cil was introduced at a four per cent level and mixed well with the ration. The mixed rations were then placed in numbered sacks and stored in a refrigerator prior to, and during each experiment. To prevent deterioration due to prolonged standing, 2000-to 2400-gram amounts of the rations were

1 Obtained from Brown Company, Berlin, New Hampshire.

Table III

Composition of diets used in Experiments I, II, and III.

Constituents			Amou	nt per	100 g	rans	of die	et.			
<u>0.055 (0.0</u>	1	2	3		4	1		2	3	4	
Experiment I (Protein- 12.07%)					Experiment II (Protein- 12.00%)						
Casein Wheat Wintok Cherokee	13.90	90.00	76.0	50 72	8.90	13.80)	90.00	76.85	75.74	
Solka-flock Corn starch Corn oil Salt mixture Vitamin " Cod-liver oil	76:10 8.00 2.00 0.20	8.00 2.00 0.20	13.4 8.0 2.0	10 1 00 1 20 2	7.10 3.00 2.00 0.20	7.89 74.31 2.00 2.00 0.20		0.60 5.94 2.00 2.00 0.20	2.29 16.86 2.00 2.00 0.20	20.26 2.00 2.00 0.20	
	1	2	3	4	Die 5	ts	6	7	8	9	
				Ex (Pro	perim tein-	ent 1 10.5	III 6%)				
Casein Wheat Wintok	12.27	85.23	78.42	80.43							
Forkedeer Stanton Str. Traveler Desoto	I				83.	88	78.42	82.18	84.34		
Andrew										83.67	
Solka-flock Corn starch Corn oil Salt mixture Vitamin " Cod-liver oil	9.62 74.10 2.00 2.00 0.20	7.78 2.98 2.00 2.00 0.20	2.78 14.77 2.00 2.00 0.20	2.68 12.90 2.00 2.00 0.20	1. 11. 2. 2.	38 23 00 00 20	1.88 15.66 2.00 2.00 0.20	1.72 12.00 2.00 2.00 0.20	11.66 2.00 2.00 0.20	3.32 90.40 2.00 2.00 0.20	

Table IV

Composition of diets used in Experiment IV

(Protein- 10.01)

Constituents		Amount per 100 grams of diet. Diets							
	1	2	33	4	5	6	7	88	9-12
Casein	11.97								
Wheat		89.40							
Neosho Date I			79.26						
Neosho Date III				74.60					
Andrew Date I					78.82				
Andrew Date III						86.66			
Forkedeer (Perk.)							78.51		
Forkedeer (L. B.)								80.21	
Tennex									68.00
Tennex + 0.5% IL-1	ysine						19.184		68.00
Tennex + 0.5% DL-n	eth.								68.00
Tennex + 0.5% of e	ach								68.00
Solka-flock	8.77	6.60	-	1.39	2.73	0.74	0.33	1.70	3.87
Corn starch	75.27	- 1	16.74	20.01	14.45	8.60	17.16	14.09	24.3.3
Corn oil	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00
Salt mixture	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00
Vitamin "	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20
Cod-liver oil									

* DL- amino acids were used at a 0.5% level with the basal Tennex ration.

made up at one time. To insure an adequate amount of the fat soluble vitamins A, D, and E, cod-liver oil, fortified with alpha tocopherol acetate was introduced in sufficient amounts to more than meet the recommended daily requirements according to Brown (4). TABLES III and IV give the percentage composition of the rations employed in each experiment.

Rat Assay

Female albino rats weighing from 40 to 60 grams were obtained from Sprague-Dawley of Madison, Wisconsin for use in the assay. They were selected at random for the different test groups and placed in separate cages in a room in which the temperature was maintained at 75° F. Adjustments were made in order that the mean weight of the groups did not vary more than two grams at the initiation of each experiment.

Food intake was restricted to ten grams per day; the rats were fed four times weekly, necessitating the feeding of 20 grams on three of the feeding days. The animals were weighed bi-weekly on the first and third feeding days. In order to secure an accurate measure of total food consumption, all refused or spilled food was recovered and weighed. This was subtracted from the total amount of food offered to determine the food intake. All of the dietary components were present in the ration as fed, save the cod-liver oil, which was introduced to each feed cup four times per week.

Each experiment was conducted over a six-week period. Fifteen animals were used in each experimental group in the first and second experiments and ten animals were employed in each lot in Experiments III and IV. Each group was designated by the number of the diet fed;

diets one and two were the casein and wheat containing diets, respectively, in each experiment. The experimental rations employed, the number of animals and the varietal or environmental factors studied for each experiment performed, appear in TABLE V.

Table V

Source of protein, number of animals used and factors studied in relation to their effect on protein quality in each experiment,

Protein source	Number of animals	Factors studied
(1	<u>Experiments I</u> Protein- 12.07 au	end II * nd 12.00%)
Casein Wheat Wintok **Cherokee	15 15 15 15 <u>Experiment III</u> (Protein- 10.56)	Variety (spring vs winter) %)
Casein Wheat Wintok Tennex Forkedeer Stanton Strain I Traveler Desoto **Andrew	10 10 10 10 10 10 10 10	Variety 6 winter and 1 spring)
	Experiment IV (Protein- 10.01)	%)
Casein Wheat	10 10	
**Neosho Date I Neosho Date III **Andrew Date I Andrew Date III	10 10 10 10	Date of planting
Forkedeer (Perk.) Forkedeer (L. B.)	10 10	Location of planting
Tennex Tennex + 0.5% DL-lysine Tennex + 0.5% DL-methionine Tennex + 0.5% of each	10 10 10 10	Amino acid supplementation

* Experiment II was a replication of the first experiment. ** Spring varieties used. Others were winter varieties.

RESULTS AND DISCUSSION

Experiments I and II

It is evident from the curves in Figure 1 that the nature of the protein fed appreciably affected growth. Animals which received casein as a source of protein made the greatest gains in weight: those which received wheat made the poorest gains, and intermediate between these control groups were the two groups of animals which received oats as the sole source of protein. Differences in response between these two groups indicated that the biological value of the protein of Wintok, a winter oat variety, was superior to that of Cherokee, a spring variety. These results were confirmed in Experiment II, a replication of Experiment I initiated one month after the termination of the first experiment. The average gain in weight by each group in these experiments over a period of six weeks is shown in TABLE VI. In the first experiment the mean gain in weight by the rats which received Wintok was 7.81 grams greater than the weight gained by the rats receiving Cherokee, although both groups consumed practically the same amount of protein. In the second experiment there was also a greater growth response to Wintok than to Cherokee, but the difference between the mean gains by the two groups was only 1.8 grams.

Data from Experiments I and II were subjected to an analysis of variance (Snedecor, 31) to determine the statistical significance of the differences in growth response. Since the total protein consumption was available, an analysis of covariance was first made to determine whether variations in growth response were due to differences in the amount of protein consumed. The analysis indicated that the differences in weight gains were not due to variation in the amount of protein consumed; on the contrary, because of the restricted feeding, the divergence in protein intake among the rats was negligible. When, by means of covariance analysis, an adjustment was made so that all animals on the various diets or "treatments" had the same protein intake, the resulting adjusted treatment means were not sufficiently different from the unadjusted means to warrant the use of the adjusted values. For this reason the total mean gain for each treatment was taken to represent the biological value of the protein under question. It has been shown repeatedly by the use of covariance in experiments which were used to assay protein quality. that the gain in weight of young growing rats may be used as the criterion to measure the quality of the protein assayed, provided the total food intake is restricted and subsequently measured. Recent investigations (13) bear out this statement.

The high growth response to the casein ration as compared to the cat rations was found to be highly significant (1% level), and the growth response to cat rations was significantly greater than the response to wheat. This was observed in Experiments I and II, and with but one exception, in each of the experiments performed. Application of the analysis of variance to Experiment I also revealed a highly significant difference (1% level) in the growth response of the rats to the two cat varities; the biological value of the protein of Wintok, the winter variety, was superior to that of the spring variety, Cherokee. In the second experiment the results indicated a trend in the same direction, but the

difference in growth response to the two varieties was not statistically significant. It was deemed highly probable that the results of the latter experiment would have likewise shown statistical significance, had the protein intakes been equalized and the over-all mean weight of fourteen rats been used, rather than fifteen. One rat on the Wintok oat ration performed very poorly, having a six-week total gain of only 36 grams as compared to 63.5 for the group mean. This gain was considerably below the next lowest value of 55 grams gained by another animal in this group, and did not represent the normal growth response to this oat protein.

It is believed, therefore, that the second experiment confirms the first, and that the winter variety, Wintok, is superior in growth promotion for the rat to the spring variety, Cherokee. It should not be inferred from the results of these two experiments that all winter varieties are superior to all spring varieties; on the other hand, from the evidence obtained in Experiments III and IV there appears to be a trend in this direction. It is further suggested that winter oats are a better choice from an agronomic standpoint (29) because the winter varieties in nearly every case yield better than do the spring varieties, offer more resistance to green-bugs and disease, and generally yield more protein per acre.







Table Vl

Mean gain in weight, mean protein intake and protein efficiency quotient in each experiment.

Protein Tot source (s	tal mean gain grams)	Total protein consumed (grams)	Protein efficiency quotient
	Experimen	τI	
Casein	83,8	48.70	1.72
Wheat	52 ₉ 5	46.88	1.12
Wintok	74.9	48.63	1.54
Cherokee	67 . 1	49.70	1.35
	Experimen	t II	
Casein	87.2	47.91	1.82
Wheat	48.9	45.60	1.07
Wintok	63,5	46.84	1.36
Cherokee	61.7	48.08	1.28
	Experimen	t III	
Casein	72.9	42.74	1.7 1
Wheat	43.9	42.36	1.04
Wintok	56.1	41.45	1.35
Tennex	55.5	41.45	1.33
Forkedeer	58.1	42.57	1.37
Stanton Strain I	53.5	42.51	1.26
Traveler	55.6	41.84	1 .33
Desoto	33.9	38.91	0 .88
Andrew	53.9	42.03	1.28
	Experimen	t IV	
Casein	77.1	40.93	1.88
Wheat	45.3	40.40	1,12
Neosho Date I	52.5	41.00	1.28
Neosho Date III	51.08	40.91	1.27
Andrew Date I	56.9	41.24	1.38
Andrew Date III	57.3	41.42	1,37
Forkedeer (Perkins)	59.0	41,52	1 42
Forkedeer (L. Blackwell)	57.0	41.56	1,37
Tennex	56.3	41.00	1.37
Tennex +0.5% DL-lysine	68.1	41.50	1.64
Tennex +0.5% DL-meth.	59.1	41.39	1.43
Tennex +0.5% each	69.1	41.16	1.68

Experiment III

The biological value of the protein of six winter oat varieties, Wintok, Traveler, Forkedeer, Desoto, Stanton Strain I, and Tennex, as well as one spring variety, Andrew, was compared in Experiment III. The mean growth curve for each group of animals, except those which received Tennex and Andrew, is shown in either Figure 2 or Figure 3. Curves were not included for these two groups because of their close proximity to the group curves of animals which received the other winter varieties described, but they are shown in Figures 4 and 6. There were no marked differences in the growth response of animals receiving Wintok, Traveler, Forkedeer, Stanton Strain I, Tennex and Andrew, respectively, as the sole source of protein. In TABLE VI it is evident that there was little difference in the mean gain of the animals receiving these diets, as well as relatively little variation in the protein efficiency quotients (PEQ). A comparison of mean gains and the PEQ obtained in all groups reveals that the growth response of animals which received the variety Desoto was even lower than that of animals which received wheat. It was surprising to find an oat protein of such apparently low biological value. Whether this was due to the fact that the sample received for the assay of this variety was slightly contaminated with some extraneous material, or that the protein of the variety was actually of inferior quality will remain for further investigation. There is no doubt, however, that the rats on the ration containing this variety received the same per cent of protein as the other groups, for each variety of oat was carefully analyzed for crude protein, and each ration was carefully prepared. It is of interest to note that when this variety is produced out of its optimum growing location, eastern and southeastern













Oklahoma, it exhibits poor yield in almost every instance; this might conceivably account for an oat protein of poor quality, since the oats used in the assay were grown in the Lake Blackwell area, in north central Oklahoma.

Analysis of variance showed that there was a highly significant variation (1% level) in the biological value of the oat proteins studied in this experiment. The growth response to Desoto was significantly lower (1% level) than the response to all other cat varieties. Growth response to the other varieties did not vary significantly.

The work of Heathcote (11) does not support the idea that there are varietal differences in the biological value of eat proteins. It is reasonable, however, to assume that there may be significant differences in the protein quality of varieties other than these studied by this investigation, as the results of the third experiment indicate. With this assumption in mind, one must not disregard the possibility of environmental factors effecting one variety favorably, while proving unfavorable for the optimum growth and maturation of other varieties.

Experiment IV

Since this experiment consisted of three studies conducted simultaneously to determine the effect of date of planting, location of planting, and amine acid supplementation on the biological value of oat proteins, each phase is discussed separately.

<u>Effect of date of planting</u>: As is indicated by the growth curves shown in Figure 4, date of seeding had no effect on the protein quality of either of the varieties. The difference between the mean gain in weight of the animals fed a ration containing Neosho Date I and the gain by

24

3.4





those fed Neosho Date III was negligible as was the differences between the respective protein efficiency quotients (TABLE VI). In the two groups of animals fed Andrew Date I and Andrew Date III, the same trend was indicated. There was noted a slight variability in growth response to the two varieties used; Andrew appeared alightly superior to Neosho (Figure 4) for growth promotion. Both varieties are reasonably well adapted to this area, and the slight superiority of the protein quality of Andrew as well as its seemingly greater palatability would tend to favor this variety in animal and poultry mutrition, provided it yields well and is disease resistant.

When the analysis of variance was conducted on the experimental data it confirmed the above observations that there was no significant difference either in the protein quality of the same variety of oats planted at different times or in the biological value of the proteins of the two varieties used (1% level).

Effect of location of planting: Location of planting did not significantly affect the protein quality of the variety Forkedeer (Figure 5). Growth response to this variety produced at one of the Oklahoma Agricultural and Mechanical College farms located in the Ferkins area, was similar to the response to the same variety produced in the Lake Blackwell area. The proximity of these two locations may have lessened the possibility of the occurrence of differences in the protein quality of this variety. It is logical to assume that location could conceivably affect the quality of oat proteins, provided there is a sufficiently wide margin of difference in the environmental conditions and for this reason it would be desirable to carry out a more extensive study from more varied locations in the state. Ivanov (15) has shown that variations in latitude could







cause considerable variation in the per cent protein of cats, barley, rye, and wheat, and it seems reasonable to believe that such variations could cause a difference in the biological value of the proteins. <u>Effect of amino-acid supplementation</u>: The effect on growth of the addition of lysine and methionine, separately and together, in a basal diet in which cats of the Tennex variety was the sole source of protein is evident in Figure 6 which presents the mean growth curves of animals receiving the supplemented and unsupplemented diets. Supplementation of the basal ration with 0.5 per cent DL- lysine resulted in an increased rate of growth. Over a period of six-weeks, the mean gain in weight by the animals which received this emino acid was 11.8 grams greater than the mean gain of animals which received the unsupplemented diet (TABLE VI), and the mean protein efficiency quotient was 0.27 greater for the former group than for the latter.

On the other hand, inclusion of 0.5 per cent DL- methionine in the basal Tennex ration had only a slight effect on growth rate; the mean gain in weight of the rats fed methionine was only 2.8 grams greater than that of the rats on the basal ration alone, and the PEQ of the supplemented group was only 0.06 greater than that for the unsupplemented group (TABLE VI). The inclusion of both lysine and methionine in the ration, each at the 0.5 per cent level, resulted in no greater growth response than was obtained by the addition of lysine alone; the mean weight gain was only one gram greater for the rats which received both supplements than for those which received lysine alone.

Analysis of variance of the data showed that the gain in weight by animals which received the lysine-supplemented ration was significantly greater (1% level) than that of animals receiving the unsupplemented

ration; the same was true for the gain made by the animals receiving both lysine and methionine. The difference between the weight gained by animals on the ration supplemented with methionine, and gain by those on the unsupplemented ration was not significant. These results indicate that the protein of the variety Tennex was definitely deficient in lysine, but that it was probably not deficient in methionine. There was only a slight indication that methionine may be the secondary limiting amino acid in cat protein as suggested by Mitchell (23); the mean gain in weight of animals which received methionine as well as lysine was somewhat greater than the gain by those which received lysine alone.

A comparison of the growth of animals receiving casein as the sole source of protein with that of those receiving the lysine supplemented Tennex ration raised the question as to why the animals which received lysine did not grow as well as those which received casein, since the two proteins differ only in their lysine content. It is suggested that differences in the digestibility of casein and the protein of cats accounts for this difference. Two groups of animals may receive different proteins at the same level, but only those amino acids which are liberated by enzymatic digestion and subsequently absorbed can contribute to the mutrition of the animal. It might even be postulated that the difference in digestibility between cat protein and casein, may conceivably lie in the presence of small amounts of anti-enzymatic factors present in varying amounts in the various variaties of cats; this had been demonstrated in raw soy-beans and other plant proteins. Unquestionably more light will be cast on the differences existing in the digestibility of plant proteins as compared to animal proteins in the future.

From a practical point of view, when and if feed grade DL-lysine becomes economically feasible, its inclusion in rations containing cereal grains would very markedly improve the biological value of their proteins, making them compare favorably with animal protein.



Figure 6

SUMMARY

Four experiments were conducted to determine the effect of variety, date and location of planting, and amino-acid supplementation on the biological value of oat proteins. In each experiment female albino rats weighing from 40 to 60 grams were fed rations which were identical in protein content, but differed in protein source. Food intake was restricted to ten grams daily and individual food consumption was determined. The animals were weighed twice each week and the tests were run for six weeks. Data obtained in each experiment were analyzed to determine the statistical significance of variations in the growth response of the rats.

The following trends were noted from the data obtained:

1. The protein of Wintok, a winter out variety, was found to have a higher biological value than the protein of Cherokee, a spring variety. In the first experiment rats which received the former variety gained 7.8 grams more weight over a period of six weeks than rats which received the latter variety, and the PEQ was 0.29 higher for Wintok than for Cherokee. Results obtained in the second experiment confirmed those obtained in the first, although the difference in weight gained was not pronounced. Variance analysis showed that the biological value of Wintok was significantly greater than that of Cherokee.

2. In Experiment III proteins of cat varieties Wintok, Tennex, Forkedeer, Traveler, Stanton Strain I, and Andrew did not differ significantly in biological value as measured by growth response. The protein quality of the variety Desoto, however, was significantly (1% level) inferior to that of the other varieties studies in the third experiment. Rats which received Desoto as the source of protein gained 10.0 grams less than rats on the control wheat ration and the PEQ of the former group was 0.16 lower than that for the latter group. In all other experiments the lowest PEQ was always obtained on the wheat ration.

Date and location of planting had no statistically significant effect on the biological value of cat proteins. Differences in growth response to the two lots of the variety Neosho seeded on two different dates was negligible as was the difference in response to the variety Andrew seeded on two different dates. There was a slight difference in the biological value of Forkedeer planted in the Perkins area and that planted in the Lake Blackwell area, but the difference was not statistically significant.

4. Supplementation of the oat protein Tenner with DL-lysine at the 0.5 per cent level resulted in an enhanced growth which was practically the same as the response obtained on a ration supplemented with both 0.5 per cent lysine and 0.5 per cent methionine. The increases in weight gained on the above rations were 11.8 grams and 12.8 grams, respectively, greater than the increase obtained in the unsupplemented ration. In each case the difference in weight gained was highly significant statistically (1% level). The addition of 0.5 per cent DLmethionine to the basal Tennex ration mediated no concomitant increase in growth response of any significance over the unsupplemented ration.

BIBLIOGRAPHY

- Association of Official Agricultural Chemists. <u>Official and Ten-</u> tative Methods of Analysis. 6th ed. 1945.
- Beadles, J. R., <u>et al</u>. The Relation Between cystine deficiency in the diet and growth of hair in the white rat. <u>J. Biol. Chem.</u>, 88:623-27 (1930).
- 3. Block, R. J., D. Bolling. <u>The Amino Acid Composition of Proteins</u> and Foods. C. G. Thomas, Springfield, Ill., 1945, p. 305.
 - Brown, R. A., and M. Sturtevant. The vitamin requirements of the growing rat. <u>Vitamins and Hormones</u>. Acedemic Press Inc., N. Y., 1949, pp. 171-199.
 - Buckner, G. K., <u>et al</u>. Feeding of young chicks on grains mixtures of high and low lysine content. <u>Am. J. Physiol</u>. 39:162-71 (1915).
 - Carrol, W. E., et al. <u>Ill. Agric. Expt. Sta. Bull 436</u>, pp. 115-70 (1937).
 - Grau, C. R., and H. J. Almquist. The methionine content of feedstuff proteins. <u>Arch. Biochem.</u>, 6:287-294 (1945).
 - Guerrant, Dutcher, <u>et al</u>. The effect of the type of carbohydrate on the synthesis of the B vitamins in the digestive tract of the rat. <u>J. Biol. Chem</u>., 110:233-243 (1935).
 - 9. Harte, R. A. <u>et al</u>. Protein assay by rat growth. <u>J. Nutri</u>., 34:363-371 (1947).
- Hartwell, G. A. The dietic value of oatmeal proteins. <u>Biochemical</u> J., 20:751-757 (1926).
- 11. Heathcote, J. G. The protein quality of oats., <u>British J. Nutri</u>., 4:145-153 (1950).
- 12. Hegsted, D. M., <u>et al</u>. Choline in the nutrition of chicks, <u>J.</u> <u>Biol. Chem</u>., 138:460 (1941).
- Hegsted, D. M. and J. Worchester. A study of the relation between protein efficiency and gain in weight on diets of constant protein content., <u>J. Nutri.</u>, 33:685-701 (1947).
- Hoagland, R. and G. G. Snider. The value of beef protein as a supplement to proteins in certain vegetable products. <u>J. Agric.</u> <u>Res</u>., 34:297 (1927).

- 15. Ivanov, N. N. The influence of geographic factors on the chemical composition of plants., <u>Ann. State Inst. Exptl. Agronomy</u>, 4:23-32 (1926), via <u>Chem. Abst.</u> 22:1381¹ (1928).
- 16. Johnson, D. W., and L. S. Palmer. The nutritive properties of protein, vitamins B and G and the germ of rye. <u>J. Agric</u>. 49:168-181 (1943).
- 17. Jones, D. Breese, <u>et al.</u> Comparative growth-promoting values of the proteins of cereal grains., <u>J. Nutri</u>., 35:639-49 (1948).
- 18. Krogh, M. and A. L. Lindberg. Nutrition experiments with Danish grain products., <u>Acta Mad. Scand</u>., Sppl. 90:134-40 (1938). via Chem. Abst., 33:204 (1939).
- 19. Marais, J. S. C., <u>et al</u>. The biological value of the proteins of oats, barley, wheat bran, and pollard., <u>Onderstepport J. Vet</u>. <u>Sci. Animal Ind</u>. 15, 205-10 (1940), via <u>Chem. Abst</u>., 36:1269 (1936).
- 20. McCollum, E. V. Am. Med. Assn., 68:1397 (1917) as cited by 27.
- 21. McCollum, E. V., <u>et al</u>. The nature of the distary deficiencies of the oat kernal., <u>J. Biol. Chem</u>., 29:342 (1917).
- 22. McCollum, E. V. <u>The Newer Knowledge of Mutrition</u>, The Macmillan Co., N. Y., p. 163 (1919).
- 23. Mitchell, H. H., and R. J. Block. Some relationships between the amino acid contents of proteins and their nutritive values for the rat., J. Biol. Chem., 163:599-619 (1946).
- 24. Mitchell, H. H. and G. G. Carman. The biological value of the nitrogen mixtures of patent white flour and animal foods. J. Biol. Chem., 68:129 (1938)
- 25. Mitchell, H. H., and D. B. Smuts. The amino acid deficiencies of beef, wheat, corn, oats and soybeans for the growth of the white rat., <u>J. Biol. Chem.</u>, 95:263 (1932).
- 26. Osborne, T. B. and L. B. Mendel. The effect of the amino acid content on the dist of the growth of chickens., <u>J. Biol. Chem.</u> 26:293-300 (1916).
- 27. Osborne, T. B. and L. B. Mendel. Mutritive value of the proteins of the barley, oat, rye and wheat kernels, <u>J. Biol. Chem</u>. 41:275-95 (1920).
- 28. Osborne, T. B., <u>et al</u>. A method of expressing numerically the growthpromoting value of proteins., <u>J. Biol. Chem</u>., 37:223 (1919).
- 29. Oswalt, Roy M. and A. M. Schlehuber. Oklahoma state-wide variety tests of wheat, oats and barley., <u>Okla. Agric. Expt. Sta</u>. <u>Bull. B-366</u> (1951).

- 30. Rose, W. C. The nutritive significance of the amino acids and certa in other related compounds., <u>Science</u>, 86:298 (1937).
- 31. Snedecor, G. W. <u>Statistical Methods</u>, 4th ed., The Iowa State College Press, Ames, Iowa. 1945. 485 pp.
- 32. Sherman, H. C. and Woods, E. J. The determination of cystine by means of feeding experiments. J. Biol. Chem. 66:29-36 (1925).
- 33. Summer, E. E. The biological value of milk and egg protein in young and mature rats. <u>J. Nutri</u>, 16:129-139. (1938).

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