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We know that much time and various expensive equipments are necessary to determine the digestive coefficient with the domestic animal. If we determine artificially the digestive coefficient in a short time and precisely, the work will be a valuable contribution to the formulation and the allowance of the ration, as well as to the nutritive value of the feed.

Although we can determine the artificial digestive coefficient of the protein by the acidified solution by HCl which contains pepsin, but the result is inconsistent with that obtained by the use of the domestic animal. Accordingly, many difficulties may be expected to trace the inconsistency to its origin.

We undertook this study to establish the artificial digestion assay, which is an analogue to the digestive condition in the digestive canal of the domestic animal.

In this paper, which is a preliminary report, we shall state the results of weighing against the power of the digestive enzyme preparations obtained by the known method from the digestive system of the domestic animal.

Methods

A. *The Digestive Enzyme Preparations*[1] :

1) Crude pepsin powder is prepared by the method employed the United States of America. The glandular layer is peeled off from the fresh stomach, finely cut, 2 times volume of 3% HCl solution is added, and after shaking

for 1 hr., it is incubated at 40°C for 24 hrs. for autolysation. The autolysed contents are filtered. The filtrate is saturated with recrystallised $(NH_4)_2SO_4$ powder at 35°C. Then, the flocculent crude pepsin salted out is centrifuged and dried under reduced pressure at a temperature not above 45°C.

2) Pancreatin powder is prepared as follows: The pancreas is finely cut, immersed in 90% ethanol and then in absolute ethanol for 24 hrs. The residue is defatted with ether, centrifuged, dried under reduced pressure at a temperature not above 45°C and then sieved to exclude the fibrous matter.

B. *Digestibility of Pepsin Powder:*

Assay for egg-albumin digestive power is made according to the method(2) used in Japan. The fresh eggs are immersed in boiling water for 8 minutes, picked up and rapidly cooled by immersion in cold water. The albumin isolated is at once rubbed twice through a clean dry, 1 mm sieve. 10 gr. of the succeeding well-mixed portion is placed in an erlenmeyer flask of about 300 cc. capacity. A mixture of 100 cc. of warm water and 0.5 cc. of conc. HCl at 50°C is poured into it and after addition of the crude pepsin powder the flask is immersed in a water bath of 50°C, shaking every 15 minutes.

C. *Digestibility of Pancreatin Powder:*

1) Assay for casein digestive power is made according as the method(3) used in the United States of America. 100 mg. of finely powdered casein, stored in our laboratory, is placed in a 50 cc. volumetric flask, and after the addition of 30 cc. of water, the flask is shaked well to bring the casein into suspension, adding exactly 1 cc. of 1/10 N NaOH soulution, and held at 40°C for 15 minutes. The contents are transfered to a glass mortar, ground well until the casein is completely dissolved, and if necessary, another 1 cc. of 1/10 N NaOH may be added. In this case, 1 cc. of 1/10 N H_2SO_4 solution should be added to regulate the condition. The casein solution when cooled is returned to the flask, in which sufficient water is added to make 50 cc. 100 mg. of pancreatin powder is dissolved in 500 cc. of water. 5 cc. of the solution is placed in a test tube, in which 2 cc. of the well-shaken pancreatin solution and 3 cc. of water are added, mixing by gentle agitation. After the test tube is immersed in a water bath of 40°C for 1 hr., 3 drops of the acetic acid mixture, containing 1 cc. of glacial acetic acid, 9 cc. of water and 10 cc. of alcohol, is added to it.

2) Assay for starch digestive power is made according to the method(3) used in the United States of America.

The percentage of moisture in the soluble starch, manufactured by Kojima Chemicals Co. LTD., is determined by drying about 500 mg., accurately weigh-

ed, at 120°C for 4 hrs. A sufficient amount of water is boiled for 10 minutes and cooled to room temperature. This water is used for all dilutions hereinafter specifying water. The mixture, containing a quantity of the soluble starch, equivalent to 3.75 gr. of dry matter and 10 cc. of water, is added, with constant stirring, to 75 cc. of water, previously heated to about 55°C and contained in a 250 cc. beaker. The remaining starch is put into the beaker containing 10 cc. of water. The contents are heated to boiled gently with constant stirring for 5 minutes. Enough water is added to make the contents weigh 100 gr. The paste is cooled to 40°C, by placing the beaker in a water bath held at 40°C. 150 mg. of pancreatin powder is suspended in 5 cc. of water in a 250 cc. beaker. This suspension is added to the starch paste and mixed well by pouring the mixture from beaker to beaker for 30 seconds, noting the time when the pancreatin suspension was first added to the starch paste. The mixture is held at a temperature of 40°C for exactly 5 minutes, stirred well and at once 0.1 cc. of this mixture is added to a previously made mixture of 0.2 cc. of 1/10 N iodine solution and 60 cc. of water at a temperature of from 23°C to 25°C.

3) Assay for milk-fat digestive power is made according to the method[1] employed in England. The yield of cream, specially made in Iwate Milk Co. LTD., was 6.4 vol. % of fresk milk, having from 1.028 to 1.032 of specific gravity at 15°C, from which cream was centrifuged at 6000 rotations per minute at 34°C. This cream is suspended in a mixture, containing 0.2 cc. of oleic acid for every 100 cc. of 1/10 N Na_2CO_3 solution, the volume ratio of which is the same as the original one of milk. Then, 10 cc. of the suspension, adjusted to pH 8.0 by the addition of several drops of the dilute acetic acid, is placed in each of 2 test tubes, in one of which 1 cc. of a solution, containing 0.1 gr. of pancreatin powder in 10 cc. of water, is added, in the other of which after boiling the solution, stated above, 1 cc. of its boiled solution is added. After the 2 test tubes are rapidly immersed in a water bath of 40°C, incubated at the same temperature for 4 hrs. each of them is titrated with 1/10 N NaOH solution after the addition of the same volume of 90 % alcohol (phenolphthalein indicator).

D. *Digestibility of Pairing Crude Pepsin Powder with Pancreatin Powder:* Assay for fibrin digestive power is made as follows:

1) Fibrin is prepared according to Hammersten's method.[4]

The blood is poured in a bottle, previously containing glass beads. When the bottle is immediately shaked violently, fibrin is separated as coagulum, which is filtered through a sack, washed first in water, then with a 0.5 % NaCl solution, and again with water, finally with alcohol and ether and dried under

reduced pressure.

2) Determination of the Digestive coefficient by the formol titration method[5, 6] :

0.1 gr. of crude pepsin powder, 1 g. of dried fibrin and 50 cc. of 0.2 % HCl solution are placed in a 200 cc. flask, which is immersed in a water bath of 40°C for 4 hrs., occasionally shaking. Then, the contents are filtered. The filtrate is neutralized with 0.1 N NaOH solution (phenolphthalein indicator), heated on a boiling water bath for 10 minutes. The resulting precipitated protein is filtered and washed. This filtrate and washings are diluted to 65 cc., in which 1 cc. of 0.1 N NaOH solution is added and after ascertaining that the solution is alkalic, 0.3 gr. of pancreatin powder is added.

The flask is immersed in a water bath of 40°C for 6 hrs., occasionally shaking, the contents of which is cooled to room temperature and filtered. The filtrate is used for the following titration. To 20 cc. of the filtrate, the indicator is added and free acidity determined by titration with 0.1 N NaOH solution, then 10 cc. of neutral formalin is added and the amino acid acidity titrated. The result (total acidity = free + amino acid acidity) is expressed in terms of glycine (the major constituent of fibrin) and the % of hydrolysed amino acid calculated.

Results

1) The yield of enzyme preparations and fibrin are given in table I.

Table I. The Yield of Crude Pepsin Powder, Pancreatin Powder and Fibrin.

Kind	Crude pepsin powder (% to the glandular layer of the stomach)	Pancreatin powder (% to the pancreas)	Fibrin (vol. % of the blood)
Cattle	3.0	7.2	0.3
Horse	5.8	9.3	0.4
Swine	7.5	15.3	0.3 (1.3*)

* only one example.

The contents of moisture, nitrogen and ash in fibrin are given in table II, where nitrogen is determined by the micro-kjeldahl method with the decomposing agent of mercury acetate and ash by ashing at a temperature of from 700°C to 800°C with the platinic crucible.

Table II. The Contents of Moisture, Nitrogen and Ash in Fibrin.

Constituent	Cattle	Horse	Swine
Moisture %	7.31	9.40	8.80
Nitrogen *	12.83	18.22	17.74
Ash *	1.83	5.41	0.88

* Shown as the % in the dry matter.

(2) The results of assay for egg-albumin digestive power are given in table III.

Table III. The Egg-albumin Digestive Power of Crude Pepsin Powder.

Kind	Sample gr.	Degree of digestion shown as % at		
		2 nd hr.	3 rd hr.	4 th hr.
Swine	0.1	90～95	100	
Horse	0.1	ca. 50	ca. 50	ca. 50
Do.	0.2	90	100	
Cattle	0.1～2.5	*	*	*
Merck's pepsin	0.1	80	100	
Pepsinum Saccharatum	0.1	60	90～95	100

* As the major portion of the substrate is difficult to be digested, the degree of digestion is unknown.

In consequence of the result given in table III, the crude pepsin powder of cattle is assayed particularly, according as the N. F. method[7] used in the United States of America.

0.2 gr. of the egg-albumin, prepared in B. of the methods stated in this paper, is placed in a 50 cc. flask, in which a mixture of 20 cc. of warm water and 0.1 cc. of conc. HCl at 50°C is added and mixed well. After addition of the crude pepsin powder, the flask is immersed in a water bath of 50°C for 2 hrs., occasionally shaking. The contents are transfered to a graduated centrifuge bottle of 25 cc. capacity. The two methods are adopted, in one of which the contents are centrifuged at 1300 or 2200 rotations per minute for 5 minutes, in the other of which the contents are kept still for 30 minutes. The results obtained are given in table IV.

Table IV. The Quantity Submerged of the Indigestible Egg-albumin (cc.) and Digestive Power (%).

Assay		2200 r. p. m.*	1300 r. p. m.*	Kept still for 30 minutes	Digestive power
1	Test[1]	4.5～5	5	5.5	ca. 2
	Check	6.5～7	7.5～8	6.7	
2	Test[2]	4	5	4.5	ca. 2
	Check	5～6.5	7.5	6.7	

1 contains 0.25 gr. of the cattle sample.
2 contains 0.50 gr. of the cattle sample.
* r. p. m. = rotation per minute.

(3) The results of assay for casein, soluble starch and milk-fat digestive

power of the pancreatin powder are given in table V.

Table V. Digestive Power of Pancreatin Powder.

Assay for	Kind	State		Power
Casein	Swine	Turbidity by the	none	+
	Cattle	acetic acid	a little turbid	±
	Horse	solution	turbid	−
	P*		do.	−
Soluble starch	Swine	Coloration by	a little yellow	+
	Cattle	the iodine	reddish yellow	±
	Horse	reaction	purple	−
	P*		do.	−
Milk-fat	Swine	Difference	0.80	+
	Cattle	of titration	0.53	+
	Horse	number (cc.)	0.70	+
	P*		0.05	−

* Pharmacopoeia japonica

1. The solubility of pancreatin powder of swine, cattle and horse was imperfect.
2. The signs of " + " " ± " and " − " indicate respectively, pass, pass doubtfully and reject for quality.

(4) The results of assay for digestible power of pairing crude pepsin powder with pancreatin powder are given in table VI.

Table VI. Digestive Coefficient %

Fibrin	Crude pepsin powder and pancreatin powder	Digestive Coefficient
Swine	Swine	25.2
	Horse	11.2
	Cattle	7.5
Horse	Swine	16.7
	Horse	9.4
	Cattle	4.6
Cattle	Swine	23.2
	Horse	8.5
	Cattle	5.4
Fibrin, prepared by Takeda Chemicals Co. LTD.	Swine	18.6
	Horse	10.0
	Cattle	5.1

Discussion

The studies[8–13] on the digestive enzyme of the domestic animal and on the methods, by which the digestive power of pepsin is approved, have been reported, but further study on the comparison among the power of the digestive enzymes of the domestic animal has not yet been made.

M. K. Horwitt[14] studied on the artiffcial digestion of the protein of green leaves, using the pepsin and the trypsin, but did not approve these digestive powers particularly.

As shown in table 1, the yields of pepsin powder, pancreatin powder and fibrin of swine are the most of the 3 kinds of domestic animal, but it may be thought, perhaps, that even if the difference among the yields is shown it indicates merely the yields obtained by the same method of preparation from the different samples.

As shown in table 2, the contents of moisture and nitrogen in fibrin show no appreciable difference among the 3 kinds of domestic animal, but the contents of ash in horse fibrin was the greatest. Each obtained fibrin is white.

As shown in table 3, the egg-albumin digestive power of swine's pepsin powder is the greatest, followed by that of the horse and cattle powder in the order named.

Yet, as shown in table 4, it is thought that as the indigestible egg-albumin is difficult to be determined when centrifuged, either its surface is inclined or it makes large blocks or its large blocks float on the liquid surface, the reading of measure by the latter method is more correct than the former.

As shown in table 6, it is noticed that the disgestive power of pairing crude pepsin powder with pancreatin powder, in spite of the kind of fibrin, swine's, horse's and cattle's power come in the order named respectively, their proportion being approximately 4, 2 and 1. From these results, it is necessary to in vestigate, whether the optimal condition of horse's or cattle's enzyme is satisfied, even if it is thought that the optimal condition of swine's enzyme is satisfi ed. R. Sasaki et al,[15] report that when the cattle fibrin is digested by the horse pepsin a temperature of from 42°C to 44°C is optimum. We obtained a better result, showing that the digestive coefficient is 10.5 % against 8.5 % obtained by the above method in this paper, when cattle fibrin is digested by the horse pepsin powder at 44°C, then by the horse pancreatin powder at 40°C and the digestive coefficient is determined by the formol titration method.

In regard to the digestive power of pancreatin powder for casein, starch and fat, by piecing it together here and there, swine's, cattle's and horse's power come in the order named respectively. It is stipulated in the Pharmacopoeia of the United States of America that the pancreatin powder is always

prepared from the pancreas of swine or cattle, so it is thought that this order is maybe correct.

The digestive power of the pancreatin powder made according to the Pharmacopoeia japonica was poor, unexpectedly. Its powder was about 2 years old after the preparation. It is noticed that the digestive power of such an old sample should be expected to be poor.

Now, as shown in table 6, the specificity is also not to be asknowleged qualitatively, with which swine fibrin is specially digestd by its own pepsin and pancreatin, horse fibrin by its own pepsin and pancreatin and so on.

The power of swine's, horse's and cattle's digestive enzymes, prepared by the known method, is expressed in figures in table 6, but it is doubtful whether it is rational to prepare the horse's or cattle's digestive enzymes by the same method as the existing, whereas this method is used for the preparation of swine's digestive enzyme. After all, we think that it is essential to investigate the optimal condition in the preparation of the digestive enzymes in regard to cattle and horse to accomplish the purpose of our study.

Summary

1) By the known method, the crude pepsin powders are prepared from the glandular layer of the stomach and the pancreatin powders from the pancreas of cattle, horse and swine.

2) The former are assayed for the egg-albumin digestive power, according to the methods of the Pharmacopoeia japonica and the N. F., and the latter for casein, starch and fat digestible power, according to the methods of the Pharmacopoeia used in the United States of America and England.

3) Each fibrin is prepared from the blood of 3 animals and the digestive coefficient is determined by the formol titration method after the fibrin is digested by pairing severally the crude pepsin powders with the pancreatin powders of the 3 animals in this order.

4) The swine's digestive power of the crude pepsin powder is the strongest followed by the horse's and cattle's power in the order named. Of the pancreatin powder, the swine's digestive power is the strongest followed by the cattle's and horse's power. From the result obtained by the determination of the digestive coefficient, the swine's digestive power is the strongest followed in the order by the horse's and cattle's power in every case, so the specificity is not to be acknowleged qualitatively, with which the digestive enzyme of the 3 domestic animals is active for the special fibrin as substrate.

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