SUMMARY
The present investigation entitled, "Studies on anorexia due to Zn-deficiency", was carried out on two animal models, mice (Mus musculus) and common carp (Cyprinus carpio). The main objective of this study was to find a correlation of accumulation of lipid in Zn-deficient animals to the low food intake in them. This was investigated by employing cytochemical and biochemical techniques.

For experimentation the animals were divided into three groups, A, B and C. Group A served as the experimental and B and C as the control groups. The animals were provided semi-synthetic purified diet. The group A was fed this diet but Zn was lacking in its mineral mixture (ZD). The group B was kept on the same semi-synthetic diet containing 300mg of zinc sulphate/300g of diet in the case of carps and 100mg of zinc sulphate/kg of diet in mice (ZS). The group C or the pair-fed (PF) group was fed ZS diet in an amount equal to that consumed by ZD group in the preceding 24h. This phase of the experiment lasted for 8 weeks and was termed as the lipid adequate phase of feeding.

At the end of this phase, the corn oil (6%) was deleted from the diet of all the previous groups and they were fed the respective diet containing 3% codliver oil as the lipid source instead of the 9% employed in lipid adequate phase of feeding. The second phase of diet treatment was termed as the lipid restricted phase and the groups were
labelled as lipid restricted Zn-supplement (LRZS), lipid restricted Zn-deficient (LRZD) and lipid restricted pair-fed (LRPF). The second phase lasted for further 8 weeks.

**FOOD INTAKE**

The daily food intake in ZD and ZS groups of mice remained similar during the early weeks of the lipid adequate phase of the feeding. After weeks 4, ZD mice group consumed only the morning ration and the evening ration was left unconsumed. Their food intake stabilized to 5g/100g in ZD group against 9g/100g of body weight in ZS group after 6 weeks onwards. The food intake in LRZD group exhibited a gradual increase and approached close to that of LRZS group after the end of the second phase of feeding.

In carps too, the food intake was similar in ZS and ZD groups for the first two weeks only. But from 3rd week onwards, the ZD carps group started consuming only the morning ration and the evening ration was left as such. Their food consumption was recorded as 0.563g in ZD group as against 1.326g/100g in ZS group on wk 8. From week 2 of the second phase onwards, the carps of LRZD group had elevated food intake. Finally at the end of week 8 of this phase, the food intake was recorded as 4% of the body weight.
Summary

The estimations of Zn in the small intestine and liver of different groups of mice and carps were made after 8 and 16 weeks of dietary treatment. The data indicated a consistent and significantly lower concentration of Zn in the intestine and liver of ZD groups of the two animals estimated after first and second phase of diet treatment.

The data is suggestive of the involvement of lipid as a cause of low food intake in animals fed lipid adequate ZD diet and not the metal itself as the food intake improved and approached near to that of lipid adequate ZS group on reducing the dietary lipid content to 3% in their diet inspite of Zn-deficiency.

BODY WEIGHTS

The data on body weights of the animals recorded its gradual loss in ZD animals compared to those of ZS and PF groups during the lipid adequate feeding phase. The mice of ZS group gained 36.41g, PF gained 16.5g while ZD lost 4.7g/100g of the body weight. The carps of ZS group gained 19.7g as against 12.18g/ and 3.16g/100g of body weight reduction in the case of ZD and PF groups, respectively.

LRZD, LRZS and LRPF mice revealed a gain in body weight by 29.09g, 11.46g and 15.35g, respectively, per 100g of body weight during the lipid restricted phase of feeding.
(wk.9-16). In carps also, the body weight in LRZD increased and approached close to LRZS at week 6 and exceeded at week 8 of the second phase of feeding.

This suggests that the loss of the body weight in lipid adequate ZD groups was associated with the lower food consumption and subsequent malabsorption of nutrients as a result of the excess of lipid in diet.

GASTROSOMATIC INDEX (GSI) AND STOMACH CLEARANCE RATE (SCR):

In order to find out the mobility of the food from stomach to intestine, the stomach clearance rate was estimated in all the groups of animals by calculating the values of gastrosomatic indices determined at 0, 3 and 6h after their morning feed.

GSI at 0 and 3h in ZD mice remained unchanged but it got reduced by 42% at 6h stage. However, in ZS and PF mice, it lessened by 47.97%, 37.03% at 3h and by 49.92% and 26.27% at 6h stages, with respect to its value obtained at 0h, respectively. Based on these values, the SCR was calculated and it revealed as 15.93%/h at 3h and 11.97%/h at 6h stage in ZD mice. However, in ZD group, it was recorded as 4.89%/h at 3h and 7.14%/h upto 6h stage. In LRZD group, the GSI declined by 48.18% at 3h and 52.89% at 6h. While GSI of
LRZS and LRPF remained almost identical to that of ZS and PF groups, respectively. The difference of SCR among LRZS, LRZD and LRPF, was insignificant.

From the above data, it is evident that a full stomach took about 9 hrs. for its complete clearance both in ZS and PF groups and 14.5 hrs. in case of ZD mice. ZD mice would consume about 32.75% less food than those of ZS group in 9 hrs. This approximately coincided with the recorded daily food intake in the two groups on 8th week of the first phase of the experiment.

The ZD carps had also similar values of GSI at 0 and 3h stages and declined by 30% at 6h stage. It diminished by 35.8% to 40% at 3h and by about 80% at 6h stage in ZS and PF groups. The SCR of ZD group was 1.12%/h at 3h and 5.63%/h at 6h stage in contrast to 11.93%/h at 3h and 13.19%/h at 6h in ZS group and 10.82%/h at 3h and 10.67%/h at 6h in PF group (P<0.001 when ZD was compared with ZS and PF groups). During the lipid restricted phase of feeding, LRZD group showed a decrease in GSI by 28.93% and 60.47% at 3 and 6h, respectively. The LRZS and LRZD groups differed insignificantly at 3 and 6h stages, respectively. The value of SCR in LRZD also increased from 1.12%/h to 9.63%/h at 3h and from 5.63%/h to 11.98%/h at 6h stage. LRZS and LRPF were slightly significantly different (P<0.05) from each other.
This implies that the food stayed like that of mice in the stomach for a longer duration in ZD carps also than in ZS and PF diets fed groups and the movement of chyme from stomach to duodenum was relatively slower in the former than in the latter two groups. On the basis of stomach clearance rate, the ZD carps could clear their stomach in 15.8h if 0.50 GSI is taken as empty stomach; Or it would consume 2.63 times less food in 6h which approximately coincided with the actual food intake values.

The results of our studies on GSI and SCR envisage a defect in stomach emptying process both in mice and carps as a possible cause of low food intake in lipid adequate ZD animals and not in LRZD animals inspite of the lack of Zn in the diet of both the diet groups. This difference seems to be contributed by the excess of lipid in the former group.

CYTOCHEMICAL OBSERVATIONS:

Since the results obtained pointed towards the involvement of lipid in anorexia in ZD animals, the fate of dietary lipid in the intestine was further studied by employing Sudan Black B technique cytochemically on the intestine at 0, 3 and 6h stages of starvation. The cytochemical analysis revealed a deep homogeneous SBB reaction in the cytoplasmic area of the enterocytes of jejunum of mice and midgut of carps at 0h stage and changed to moderate
intensity at 3h stage. It was almost negative at 6h stage in ZS, PF, LRZD, LRZS and LRPF groups. In ZD animals, however, the reaction appeared unchanged at 3h stage and slightly less intense at 6h stage compared to that at 0h stage. This differential behaviour of SBB reaction in ZD with respect to that of ZS, PF, LRZD, LRZS and LRPF groups indicated that the lipid were cleared from the enterocytes in less than 6h of feeding in the latter groups while it stayed in them and took relatively longer time for its clearance to intercellular spaces/lacteal in the former group of both the animal models.

The presence of lipid in enterocytes has been reported to impose an inhibitory effect on gastric secretion and stomach emptying process through feedback mechanism. A similar mechanism seems to operate under Zn-deficiency condition also. From the data, therefore, it is concluded that the loss of appetite in ZD groups basically stems from the defects in lipid absorption which drastically reduces the movement of food from the stomach to the next part of the alimentary canal and the animals remain in a state of satiety for a longer duration. A reduction of lipid in diet eliminates the inhibitory factor and thus promotes food intake leading to body weight gain.
Further, a reduction in dietary lipid content in ZD animals promotes both food intake and gain in body wt. This implies that the reduction in body wt. in lipid adequate ZD groups is also associated with the malabsorption of lipid. To confirm this, \( ^{14} \)C-glucose uptake in intestine of both the animal models after 3h of their morning feed was investigated.

The \( ^{14} \)C-glucose concentration in intestine exhibited 73.66% and 67.90% reduction in the ZD mice compared to that of ZS (\( P<0.001 \)) and PF (\( P<0.001 \)) groups, respectively. But ZS and PF groups had an insignificant difference of the concentration of labelled glucose between them. The LRZD group concentrated \( ^{14} \)C-glucose in the intestine almost identical to that of the lipid adequate ZS mice. It was insignificantly different among LRZS, LRZD and LRPF mice.

The lipid adequate ZD carps also revealed 34.78% and 31.25% reduction in the concentration of labelled glucose compared to ZS (\( P<0.001 \)) and PF (\( P<0.001 \)) groups, respectively. And the difference was insignificant when the concentration of \( ^{14} \)C-glucose was compared among LRZS, LRZD and LRPF carps.
These results suggest that the reduction of $^{14}$C-glucose uptake in the intestine was associated with the lipid deposition in the enterocytes under Zn-deficiency condition. The presence of inordinate accumulation in enterocytes, perhaps provided a resistance to the inflow of the labelled glucose uptake. A reduction of lipid in diet eliminated this interfering lipoidal factor in LRZD group that led to the improvement of $^{14}$C-glucose uptake in the intestine almost equal to that of ZS groups.

**BIOCHEMICAL ANALYSIS:**

**TOTAL LIPID, TRIGLYCERIDE, PHOSPHOLIPID & CHOLESTEROL:**

**INTESTINE:**

Biochemical analysis of the intestine for total lipid, triglyceride, phospholipid and cholesterol revealed their significantly higher concentration in ZD animals compared to those of ZS and PF groups at 0, 3 and 6h stages of starvation in carps as well as mice. But the difference of its concentration between LRZS and LRZD and LRPF was insignificant. The data supports the cytochemical observations that the transportation of lipid from the intestine to the intercellular spaces or lacteal was slower in lipid adequate ZD animals compared to the lipid adequate ZS and PF groups. The significant difference of their levels between
LRZD and lipid adequate ZS groups was evidently because of the reduction of lipid in diet.

**LIVER:**

Contrary to the intestine, the liver of the lipid adequate ZD groups of carps and mice contained lesser amount of the above three types of lipid compared to ZS and PF groups estimated at 0, 3 and 6h stages. Their reduction in the liver of ZD group evidently was the result of lower turnover rate of absorbed lipid from the intestine. The lipid restricted diet groups had their levels significantly lower in their liver in contrast to that of their lipid adequate counterparts because of the presence of lesser amount of lipid in their diet. However, their difference amongst the lipid restricted diet groups was insignificant.

This envisages that the absorption mechanism of lipid is enhanced by the presence of Zn in diet if the diet contains lipid in excess of 3%. The lack of Zn in lipid rich diet lowers the lipid absorption by inducing defects in the transport from enterocytes to the body fluids leading to anorexia and growth retardation.

**TOTAL TRICHLOROACETIC ACID PRECIPITABLE PROTEIN:**

**INTESTINE:**

ZD groups of mice and carps recorded depressed

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levels of protein in their intestine in contrast to those of respective control groups. The two groups, ZS and PF, differed insignificantly for the protein level in the two animals. However, the level was insignificantly different among the three lipid restricted diet groups of mice. In carps, LRZS & LRZD and LRZS & LRPF had slightly significant difference between them. On an overall basis, the concentration of protein was lesser in the lipid restricted diet groups compared to their respective lipid adequate diet groups.

**LIVER:**

The level of protein in the liver of different diet groups depicted an identical trend as that of the intestine. Significantly lesser concentration of protein was recorded in the liver of ZD animals against ZS and PF groups of mice as well as carps. The three lipid restricted diet groups had an insignificant difference amongst them in both the animals.

The above data, therefore, suggest that the excess of lipid in the diet raises the protein content of the liver of three lipid adequate animal groups relative to the lipid restricted diet groups. The difference in the level of Zn in LRZS and LRZD diets, however, did not result in signifi-
cant difference of protein concentration in the liver aswell as intestine of lipid restricted diet fed animals. This, therefore, contradicts the essentiality of Zn in protein synthesis. The higher level of protein in lipid adequate Zn-supplement groups compared to lipid restricted diet group could be attributed to the role of lipid as 'protein sparing' factor.

GLUTAMATE OXALOACETATE TRANSAMINASE & GLUTAMATE PYRUVATE TRANSAMINASE :

INTESTINE

The intestine of lipid adequate ZD group of mice and carps revealed a significant fall in the activity of the two transaminases compared to their two control groups. However, there was an insignificant difference of the activity of the enzymes between ZS and PF groups. The three lipid restricted diet groups of the two animals had an insignificant difference in the level of their activities.

LIVER:

The activities of GOT and GPT were significantly reduced in the liver of lipid adequate ZD mice and carps compared to those of their two control groups. ZS and PF groups differed insignificantly from each other in their activities. There was also an insignificant difference amongst the three lipid restricted diet groups of
mice and carps as well.

The two transaminases, GOT and GPT, have a significant role to play in the synthesis of protein. The lower level of their recorded activities in lipid adequate ZD animals and the reversal of the enzyme activities to the level of control in LRZD group suggests inanition as a cause of their depressed activities in lipid adequate ZD group rather than Zn itself.

NUCLEIC ACIDS:

INTESTINE:
The lipid adequate ZD group of mice and carps depicted a significant reduction in DNA & RNA concentrations compared to their respective control groups. The nucleic acid concentrations in ZS and PF differed insignificantly.

During the lipid restricted phase of feeding, the nucleic acids concentration was identical in three groups. LRZD fed groups had increased concentration compared to the respective lipid adequate ZD group and the rise in their concentration was very close to that of lipid adequate ZS group.

LIVER:

In liver also, the RNA concentration was signifi-
cantly less in lipid adequate ZD groups of mice and carps compared to their respective control groups while the difference was insignificant between ZS and PF animals. The LRZD group possessed its higher concentration compared to lipid adequate ZD fed animals and was insignificantly different vis-a-vis the level of lipid adequate ZS and PF groups.

However, the level of DNA concentration did not fluctuate significantly in the liver of experimental groups of the two animal models with respect to ZS and PF groups and to LRZS and LRPF groups.

The data evidently reveal that the level of concentration of nucleic acids fell significantly in the intestine of ZD groups when the lipid are in excess. But their levels elevated close to that of their respective lipid adequate ZS group on feeding them lipid restricted diet. Similar trend also existed for RNA, and not for DNA, in the liver of the experimental animals. This observation provides evidence that the depression of nucleic acids in lipid adequate ZD group is secondary to the lipid malabsorption and not due to the lack of Zn in tissues or diet.
GLYCOGEN:

INTESTINE:
The concentration of glycogen depicted its lessened level in lipid adequate ZD groups of mice and carps against their respective control groups. In ZS and PF groups, it remained insignificantly different from each other.

During the lipid restricted phase of feeding, however, the LRZD groups of the two animals had contrasting results. The LRZD mice possessed a significantly reduced level of glycogen, while LRZD carps had its significantly higher level compared to their respective lipid adequate ZS groups. The LRZD, LRZS, and LRPF did not differ significantly in the concentration when compared amongst one another.

LIVER:
The lipid adequate ZD mice exhibited a fall in glycogen concentration in its liver vis-a-vis that of control groups; while ZS and PF groups differed insignificantly form each other.

Contrary to mice, the ZD carps contained higher level of glycogen compared to ZS and PF groups. The level was even higher in ZS group compared to that of PF group.
The LRZD group displayed significant increase of glycogen in mice and its significant reduction in the carps against their respective lipid adequate ZS groups. However, their respective LRZD, LRZS and LRPF did not differ significantly.

**GLUCOSE-6-PHOSPHATASE, FRUCTOSE-1,6-DIPHOSPHATASE, ADENOSINE TRIPHOSPHATASE:**

**INTESTINE:**

The activities of these three enzymes were recorded lower in the lipid adequate groups of mice and carps against their respective control groups. However, an insignificant difference of their activities existed between ZS and PF groups for both the animals.

Three lipid restricted diet groups of each of mice and carps contained comparable levels of activities of these enzymes, difference of the respective enzyme activity in both the animal models being insignificant.

The activity of the three enzymes, G-6-Pase, F-1,6-DPase and ATPase, in the intestine of LRZD group were close to that of lipid adequate ZS mice as well as carps groups, respectively; their difference being insignificant between the two diet groups.

**LIVER:**

A similar activity response of these three
phosphatases in the liver with respect to the intestine of different diet groups of mice and carps was noticed during the first and second phase of the experiment.

The lower activities of the above enzymes in the intestine and liver of the experimental lipid adequate ZD group and their elevation following lipid restricted ZD diet again points towards the possible role of low substrate concentration effect on the syntheses of these enzymes rather than the essentiality of Zn in them.

ALKALINE AND ACID PHOSPHATASES:

INTESTINE:

A reduced activity of alkaline phosphatase was recorded in the intestine of lipid adequate ZD group of mice and carps compared to their respective control groups; while an insignificant difference existed in its activity between ZS and PF groups. Their activities elevated in LRZD diet group and was insignificantly different from that of lipid adequate ZS group.

Contrary to the activity of AKP, the ACP enzyme activity in lipid adequate ZD group was increased than those of ZS and PF groups in both the animal types. The LRZD group of mice exhibited significant reduction in the activity of ACP against that of lipid adequate ZD group.
Summary

LIVER:

The activity of AKP and ACP enzymes in the liver of lipid adequate ZD group was recorded significantly depressed than those of ZS and PF control groups for mice and carps, both. But their difference was insignificant between ZS and PF groups. The three lipid restricted diet groups recorded a comparable level of the activities of AKP and ACP, between them for both the animal types.

LACTATE DEHYDROGENASE & SUCCINIC DEHYDROGENASE:

INTESTINE:

In lipid adequate ZD group the intestine displayed experienced a significant reduction in the activity of LDH and SDH enzymes compared to those of two control groups in both animal types.

The lipid restricted diet groups, on the other hand, possessed an insignificant difference in their activities amongst themselves.

LIVER:

The lipid adequate ZD mice and carps underwent a significant reduction in the level of LDH activity in their liver compared to those of their respective control groups. ZS and PF, however, had an insignificant difference. The three lipid restricted diet groups differed insignificantly from one another.
Summary

The recorded lower concentration of DNA, RNA, protein, glycogen, GOT, GPT, G-6-Pase, F-1,6-diPase, ATPase, ACP, AKP, LDH and SDH and their levels in LRZD groups almost similar to lipid adequate ZS groups, is a response to lower substrate concentration effect of nutrients linked with lipid malabsorption rather than the essentiality of Zn in their syntheses/metabolism.