Assessment And Correlation Study Of Macro And Micro Nutrients In Feeds Manufactured In Various Processing Units. At Namakkal District.

By

P. Banumathi

A DISSERTATION SUBMITTED TO THE AVINASHILINGAM INSTITUTE FOR HOME SCIENCE AND HIGHER EDUCATION FOR WOMEN - DEEMED UNIVERSITY, COIMBATORE - 641 043

IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE DEGREE OF

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Signature of the
Guide

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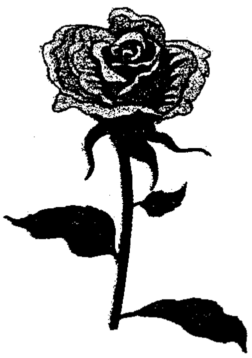

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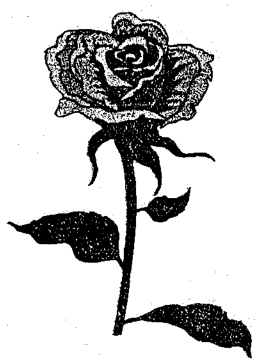


INTRODUCTION

CHAPTER I

INTRODUCTION AND OBJECTIVES

1. Introduction

In India, Namakkal region of Tamil Nadu stands second in egg production while Andrapradesh being the first. The layer population in this area has grown at an incredible pace and reached to about 18 millions and are being reared in more than 6900 commercial units.

Egg shell breakage brings in major economic losses to the poultry industry. Roland (1977) estimated that 4% of eggs are broken because of shell defects. Egg shell breakage is caused by nutritional, managemental, genetic and environmental factors. Of these, the nutritional factors namely variations in levels of calcium and phosphorus contents in the diet appear to be the main cause.

1.1 Macro and Micro nutrients

Analysis of poultry tissue or eggs, about every chemical element would be found some essential; others not. By essential, is meant not only for growth but other aspects of the performance of the birds as well. Certain essential elements, present in large amounts than others, are called 'major' or macro nutrients. Some needed in very small amounts are termed 'trace' minerals. The mineral part is inorganic (sand and silica) and remains after ashing the product. Those needed in substantial amounts are calcium, phosphorus, magnesium, sodium, potassium, and sulphur. Examples of those needed in very small or trace amounts are copper, cobalt, iodine, manganese, zinc, fluorine, molybdenum and selenium. There are others that are there in trace amounts, but are not essential to the performance of birds – aluminum, arsenic, nickel and silica. Inorganic nutrients or minerals should not be treated as single ingredients. They are interrelated just as are the vitamins and proteins. For example, calcium and phosphorus should be in definite relationship in the feed for formation of bone in the bird.

Calcium and magnesium are necessary for nerve cell function. Three minerals that are most likely to be critical in feeds are calcium, iron and iodine.

Calcium and phosphorus are vitally concerned with each other in feeds, since they are closely interrelated. A deficiency or an over abundance of one may interfere with the proper utilization of the other. Calcium and phosphorus must be in the feed in sufficient amounts and the elements must be present in forms that are efficiently digested and utilized. There is usually little concern with calcium in this regard. Calcium is seldom found to be deficient and there is no problem with respect to its biological value. Lime stone, pure calcium carbonate, calcium sulphate and calcium gluconate, bonemeal, defluorinated phosphate, and dicalcium phosphate all have essentially equal availability of calcium. Feed stuffs of vegetable origin are also generally considered as having fully available calcium.

This is not the case with phosphorus. Sources vary widely in amount of the available phosphorus. Cereal phosphorus is inadequately utilized by poultry. For example, chicks and turkeys from 8 to 20 weeks of age do not utilize phytin phosphorus to any great extent. In addition, inorganic phosphates differ widely in biological value. Thus, total phosphorus can be a misleading term.

1.2 Calcium

1.2.1 Importance of Calcium

The calcium requirement for maximum shell thickness is greater than for maximum egg production and as shell thickness is related to strength, the requirement quoted is for maximum or near maximum thickness. Most of the results available show a continuing increase in thickness with all increases in intake, even at 5 g or more daily (ARC,1975), but there is some evidence that voluntary feed intake may be depressed when diets containing high levels of calcium are given (Scott, Hull and Mullenboff,1971). The optimum daily intake is about 4.0 g but Simons (1986) suggested amounts related to the weight of egg produced daily per bird, that is 3.8 g calcium for 50 g of egg and 4.5 g for 60 g.

Among several dietary factors affecting shell quality, calcium is the most important one. A low dietary intake of calcium had been found to cause a reduction in the thickness of the shell. The amount of calcium that a hen absorbs and retains and the skeletal calcium available for shell calcification are said to decrease with age. The increase in egg shell weight is an important contributing factor to the decrease of shell strength as age advances.

Among the different minerals, calcium occurs in the highest amounts in the body. About 99% of the calcium were present in the skeleton and the remaining 1 % in soft tissues. The body of the infant at birth contains about 27.5 g and that of an adult human 1000 to 1200 g of calcium.

1.2.2 Functions of Calcium (Swaminathan , M., 1989)

The important physiological functions of calcium are

1. It is essential for the formation of bone and teeth, in which about 99% of the total body calcium is found in additi̇on it is an essential constituent of living cells and tissue fluids. Bone ash containing approximately 36% calcium, 17% phosphorus and 1 % magnesium.
2. It is essential for the clotting of blood. In blood the element occurs in the plasma. The plasma of mammals usually contains from 8 to 12 mg calcium per 100 ml, although that of laying hens contains more (between 300 and 400 mg/l).
3. It regulates the permeability of capillary walls.
4. It is essential for the contraction of the heart and muscle.
5. It regulates the excitability of nerve fibre and nerve centres.
6. It acts as an activator for the enzyme rennin present in the gastric juice.
7. Calcium in combination with intracellular receptor protein cal-modulin takes part in several enzyme reactions.

Calcium is present in plasma in 3 forms

(i) Ionized calcium

(ii) Protein bound calcium

(iii) Complexed calcium

In addition to calcium, other microelements like Zn, Mn, Mg, Fe and Cu etc. also precipitated.

1.2.3. Source of Calcium (Donald,P.Mc., R.A.Edwards, and J.F.Greehalgh)

Milk and green leafy crops, especially legumes, are good sources of calcium; cereals and roots are poor sources. Animal by-products containing bone such as fish meal and meat and bone meal are excellent sources. Mineral supplements which are frequently given to farm animals, especially lactating animals and laying hens, include ground limestone, steamed bone flour and dicalcium phosphate. If rock calcium phosphate is given to animals it is important to ensure that fluorine is absent, as otherwise this supplement may be toxic.

1.2.4. Toxicity(Jonathan.M.Naylor,1989)

Calcium has low toxicity. High concentrations in hay interfere with the absorption of other minerals.

1.3. Phosphorus

1.3.1. Importance of phosphorus

Phosphorus is a critical nutrient in the diet of laying hens. The source of phosphate, particles size and its diet levels are of particular interest. Dicalcium phosphate is used more often in diets for layers. Dietary phosphorus appears to have a bisphasic effect on egg shell quality. An inadequate level of phosphorus in the diet found to reduce egg shell quality and a high level of dietary phosphorus also found to have detrimental effects..

Phosphorus is a critical and highly expensive mineral in poultry feeding and it plays a vital role in cell oxidative phosphorylation, egg shell formation, skeletal development etc. Majority of phosphorus in plant source of feed ingredients is in the form of 'phytin' which is not available to the chicken. The free phosphorus present in plant, animal and inorganic source is readily available to the bird is called as available phosphorus (S.V. Rama Rao, 1997).

Phosphorus is an important element for shell formation, not because egg shell contains much phosphorus (there is about 100 times as much calcium as phosphorus in egg shell), but because of the special relationship between calcium and phosphorus in bone formation. Calcium is stored in the skeleton probably almost entirely as calcium phosphate and therefore synthesis of medullary bone requires dietary phosphorus. This phosphorus is however involved in an essentially 'furtile' process, because if the calcium is used for shell formation the phosphorus must be excreted.

Inorganic phosphorus: phosphorus is present as calcium phosphate in bones and teeth and as phosphates of sodium and potassium in soft tissues and body fluids.

Organic phosphorus: Phosphoric acid in combination with some organic compounds plays an important role in metabolism. These include adenylic acid, phosphate esters of monosaccharides, phosphatids, nucleic acid, nucleoproteins, Co-enzymes containing B-vitamins etc.

1.3.2. Functions of phosphorus(Swaminathan., M,1989)

The important functions are as follows

1. Phosphorus is necessary for the formation of bone and teeth.

2. It is necessary for the formation of phospholipids - lecithin and cephalin which are integral parts of cell structure and also act as intermediate in fat transport and metabolism.

3. It is essential for carbohydrate metabolism as phosphorylation glycogen requires organic phosphorus and phosphoric esters like adenyl acid, adenylphyrophosphate and creatine phosphate.

4. It is a constituent of certain co-enzymes, e.g., co-enzyme I and co-carboxylase which take part in the enzyme systems concerned in the oxidation of carbohydrates, fats and proteins.

5. It is essential constituents of nucleic acid and nucleo-proteins which are integral parts of the cell nuclei.

TABLE – 1 1.3.3 Source of phosphorus

S. No	*Food Stuffs*	*Phosphorus (g/100 gm)*
1	Cereals, Millets, Pulses, nuts and oilseeds (rich in phytate phosphorus)	0.20 to 0.65
2	Dried fish	1.2 to 1.3
3	Milk powder, full fat	0.76 to 0.82
4	Milk powder, skimmed	1.0 to 1.1
5	Meat, fish and eggs	0.31 to 0.41
6	Milk, cow's or buffalo	0.09 to 0.11

1.3.4. Toxicity (Jonathan.M.Naylor., 1989)

High phosphorus diets may suppress the absorption of calcium and can predispose to urolithiasis.

TABLE - 2 Roles played and deficieny symptoms of various elements

(Mc Donald and Edwards).

S. No	*Element*	*Deficiency*	*Symptoms*
1	Calcium	Rickets	Misshapen bones enlargements of the joints, lameness and stiffness.
		Osteomalacia	Bones become weak and are easily broken. In hens, soft beak and bone, retarded growth and bowed legs are noticed. The eggs have thin shells and egg production may be reduced.
		Milk fever	Extreme paralysis and unconsciousness commonly occurs in dairy cows
2	Phosphorus	Anorexia	Death, particularly in laying hens, will occur when phosphorus is deficient.
		Fragile bones	Weight loss, emaciation, stiffness, reduced milk production.
		Rickets	Misshapen bone enlargements of the joints, lameness and stiffness.

1.4 Inter relation between minerals

Historically one of the first relationships of nutritional significance was observed between calcium and phosphorus, calcium utilization is favorably influenced by an adequate level of dietary vitamin-D and phosphorus. Diets high in protein significantly favour the absorption of both calcium and magnesium. Since a slightly acetic intestinal pH favorably influences the absorption of calcium, lactose (which promotes the establishment of an aciduric bacterial flora) is an important dietary factor in calcium utilization. In order to be utilized properly, calcium and phosphorus must be in the ration in a proper ratio. Excess calcium interferes with the utilization of zinc, manganese and other nutrients.

In the young growing bird calcification requires intake of calcium, phosphorus and vitamin-D. Certain fatty acids exert a slight antirachitic value, thus reducing the requirements of vitamin-D. Proper balance of calcium and phosphorus minimizes the vitamin-D requirement. Similarly an excess of vitamin-D permits calcification with borderline mineral levels or with an adverse ratio of calcium and phosphorus. Thus, it is impossible to state a specific requirement for one of these nutrients without defining the levels of the other two. Elemental sulfur interferes with the absorption of vitamin-D and probably other factors. Large amounts of iron, manganese and other minerals from highly insoluble phosphates and thus cause rickets. Phosphorus, present in most plant sources as a phytate, is only partially utilized for bone formation but extra vitamin-D improves its availability.

TABLE –3 Calcium, phosphorus content of certain mineral supplements(Philip.J.Schaible;1976)

Supplement	*Ca %*	*P%*
Calcium supplements 1) Oyster shell, finely ground.	38	-
2) High grade limestone	38	-
3) Calcium carbonate	40	-
4) Gypsum	25	-
5) Wood ashes	21	-
Phosphorus supplements 1) Steamed bone meal	24	12
2) Special steamed bone meal	26	13
3) Raw bone meal	22	10.5
4) Precipitated bone flour	25	16.6
5) Spent bone black	28	13
6) Mono calcium Phosphate	20	21
7) Dicalcium Phosphate	24	18.5
8) Dicalcium Phosphate (feed grade)	26	20.5
9) Tri calcium phosphate (tech, grade)	38	18
10) Phosphate curacao Island	35	15
11) Monosodium phosphate	-	22.5
12) Disodium phosphate	-	8.7
13) Defluorinate rock phosphate	33	18
14) Phosphate limestone	36	4.5

Operationalisation of basic concepts

Layer Mash (LM): A balanced feed for layer type chickens from 20 to 80 weeks.

Grower Mash (GM): A balanced feed for growers from 8 to 20 weeks of age.

Chick Mash (CM): A balanced feed for chicks from dry old to 8 weeks.

Broiler Starter Mash (BSM): A balanced feed for broiler type chicken from day old to 6 weeks.

Broiler Finisher Mash (BFM): A balanced feed for broiler type chicken from day old to 6 weeks.

Egg quality

Egg quality is based on those characteristic of an egg that affect its acceptability to the consumer. Egg shell quality depends on several factors, viz environment, genetics, physiology and nutrition. Nutrition plays a crucial role in influencing both the quality and quantity of egg shell. The egg shell contains about 2.2 g of calcium and 20 mg of phosphorus. Part of the calcium is contributed by bone resorption and the consequent release of phosphorus is excreted (Taylor and (Hertelendy, 1961).

Shell quality

Egg shell quality is used as a convenient term for the attributes measured by specific gravity, deformation, breaking strength, shell mass and density and in this sense relates to the mineral components.

Abbreviations used

Ca	- Calcium	LM	- Layer Mash	TA	- Total Ash
P	- Phosphorus	GM	- Grower Mash	CM	- Chick Mash
M	- Moisture	BSM	- Broiler Starter Mash		
SS	- Sand and Silica	BFM	- Broiler Finisher Mash		

ANSA -1-amino-2-naphthol-4-sulphonic acid

2 *Objectives*

- To estimate the amount of macro nutrients such as calcium and phosphorus and micro nutrients such as sand and silica, total ash and moisture present in different processing units at Namakkal district.
- To study the multiple correlation between macro nutrients (Ca and P) and micro nutrients (TA, SS and M).
- To carry out correlation study between the macro and micro nutrients with different feeds such as LM, GM, CM, BSM and BFM.
- To quantify the Ca : P ratio in various feeds like LM, GM, CM, BSM and BFM
- To analyse the maximum, minimum and mean values of macro nutrients and micro nutrients present in LM, GM, CM, BSM and BFM
- To learn regression analysis of macro and micro nutrients
- To bring out the importance of Ca and P present in various feeds

REVIEW OF LITERATURE

CHAPTER II

REVIEW OF LITERATURE

2.1 Feeding Programs (Partrick Schaible, 1980)

An understanding of feeding programs and feeding methods is important to have before formulating rations. Many programs and methods are used throughout the poultry industry, often feeding methods are functions of the size, bread and purpose of the flock.

The first ground rations (Mashes) were formulated for layers about 1980, by united States investigators who based their formulations on what layers were consumed when various feed stuffs were fed free choice (cafeteria style). This mash was fed as a supplement to grain, pasture and oyster – shell.

All mash feeding came into being in 1920, when hatcheries and breeding flocks were being developed in small rural farm towns and fencing of poultry became necessary. Following this all mash, feed a free-choice mash-grain program was developed. This saved about 50% of the grinding costs.

The development of mechanical feeders led to a complete change in feeding.

There are many ways to classify feeding programs, however, the one often used is based on formulated manufactured diets. There are

(1) The all-mash complete program

(2) The mash-grain program

(3) The concentrate program

2.2 Calcium

Shell strength of eggs from meat type hens increases as calcium level is increased (Mehring, 1965). Egg production and hatchability of meat type hens on litter were not improved by feeding more than 3.91 g of calcium per hen daily

(Wilson *et al.*, 1980). One of the best determinants of calcium adequacy for breeder hens is egg specific gravity; eggs should have a specific gravity of 1.080 or greater for optimal hatchability (Mc Daniel *et al.*, 1979).

Balnave (1988) indicated that laying hens require approximately 35 g calcium per diet to maximise the production of eggs with good shell quality.

Roland *et al.*, (1996) reported that increasing calcium level increased the egg production and they also recommended calcium intake should range from 3.0 g to 4.2 g per hen per day from 21 weeks to 32 weeks of age.

Outerhout (1980) found that egg weight was highly significant and inversely proportional to the dietary calcium level.

The translation of the daily intake of calcium to a proportion of calcium in the diet clearly requires knowledge of feed intake and the value is 35.0–36.5 g/kg and are based on an intake of 110 g/day when production is high, but when the daily intake of the flock differs fairly widely from 110 g it will be desirable to maintain an overall intake close to 4 g/day by adjusting the proportion of calcium in the diet. Differences in requirement within a flock will be met in some measure by comparable differences in intake of feed.

The influence of source of calcium carbonate and of particle size on shell quality has been studied in numerous experiments. Results of early studies cannot be readily interpreted because comparisons were made between limestone and oyster shell of different particle size, when particle size was controlled most experiments showed no difference between limestone and oyster shell (Roland, 1986). Very few studies has the effect of particle size with a single source been determined, but the evidence on particle size shows that shell strength is improved, without affecting production or feed efficiency when grits contribute 67-75% of the total supplementary calcium (Scott, Hull and Mullenhoff, 1971 Kuhl and Krueger, 1982). There is little evidence at present to indicate the precise particle size or mix of particle sizes that will give maximum shell quality.

Georgievskii *et al* (1987) reported that hens fed with limestone as calcium source had greater shell thickness as compared to hens given a based diet having calcium carbonate.

2.3 Phosphorus

Daghir *et al.* (1985) stated that hens required a minimum of 0.25% available phosphorus for better egg production and they also concluded that for better egg production the available phosphorus should not be less than 0.25% in the diet.

Shivazad *et al* (1993) conducted a feeding experiment with laying hens using Iranian rock phosphate and reported that when it was added to the diet replacing dicalcium phosphate, it increased the average egg production.

Hartel (1989) found that a higher rate of mortality in layers fed on low phosphorus (3.2 g phosphorus per kg of feed) and lower rate of mortality by of addition of 1 g phosphorus per kg in the diet over and above 3.2 g.

Increasing phosphorus in the diet from 0.55 to 0.90% had no significant effect on shell weight (Abdullah *et al.*, 1988).

Daghir *et al* (1985) suggested that available phosphorus above 0.35% reduce the thickness of the shell.

Hartel (1989) observed that there was a significant improvement in shell thickness when hens were fed with lower concentration of phosphorus (3.2 g phosphorus per kg) in the diet.

Vandepopaliere and Lyons (1992) reported that hens fed with 0.4% total phosphorus in the form of defluorinated phosphate produced significantly greater number of thin shelled eggs than when fed with dicalcium phosphate.

Dietary phosphor is normally present as both inorganic and organic phosphorus. The inorganic phosphorus can be considered to be 100% available, but the organic phosphorus which is linked to phytin, has a very low availability to the birds.

Egg production was improved numerically by feeding 7/8 mg total phosphorus, requirements for hens maintained in cages may be significantly greater than for hens on litter floors (Harms *et al.*, 1961; Singsen *et al.*, 1962, Harms *et al.*, 1984).

In most experiments with broilers in which phosphorus requirements have been determined the source of supplementary calcium in the diets was precipitated chalk or ground limestone of an unspecified particle size. But it has now been shown that the available phosphorus requirements of chicks vary according to particle size of the calcium supplements.

The requirement of phosphorus for the laying hen has been studied closely in recent years, stimulated by the rising cost of phosphate and the possibility that earlier values were unnecessarily high, 350 mg/day non-phytate phosphorus. Low phosphate intakes may also have a beneficial effect on shell quality since increase in plasma phosphate cause a decline in egg specific gravity (Boorman, Volychook and Belyavin, 1985). In some experiments intake of non-phytate phosphorus below 200 mg/day throughout the laying period gave as good shell quality as larger amounts (Owings, sell and Balloun, 1977, Mikaelian and sell, 1981). But a value around 50 mg/day decreased egg production in an experiment of Rodriguez, Owings and Sell (1984).

Hartel (1989) observed that there was a significant improvement in shell thickness when hens were fed with lower concentration of phosphorus in the diet.

2.4 Calcium : Phosphorus ratio (Philip. J, Schaible, 1976)

In giving calcium supplements to animals it is important to consider the calcium: phosphorus ratio of the diet, since an abnormal ratio may be as harmful as a deficiency of either element in the diet. The calcium: phosphorus ratio considered most suitable for farm animals other than poultry is generally within the range 1:1 to 2:1 although there is evidence which suggests that ruminants can tolerable rather higher ratios. The proportion of calcium for laying hens is much larger, since they require great amounts of the element for egg shell production. The calcium is usually given to laying hens as ground limestone mixed with the diet or alternatively calcareous grit may be given adlibitum

The national research council recommends a calcium-phosphorus ratio of 1.67:1 for broilers, based on total phosphorus is used the ratio becomes 2.2:1. To be most valuable, requirements for phosphorus should be expressed as available phosphorus rather than total or inorganic phosphorus. Under practical conditions, broiler rations should contain a minimum of 0.45 % available phosphorus which means roughly 0.7% total phosphorus, as 0.8% total calcium. Starting poultry should receive at least 1% calcium and 0.5% available phosphorus which calculates to 0.9% total phosphorus.

In growing turkeys, a minimum of 1% calcium is suggested with available phosphorus content of 0.5% or a total phosphorus of 0.8%. Energy contents of the ration influents the requirements for both calcium and phosphorus. The National Research Council suggests a calcium level for laying hens of 3.75% which level may not be sufficient for old hens kept in cages during hot weather. Most major feed compositions today put out rations containing 3 to 3.5% during winter months and from 3.5 to 4% in summer time.

2.5 Moisture: (Berguquist .D.H, 1986)

The vapour pressure of dehydrated whole eggs in a low moisture range 0.5 to 5.5%, was the subject of early studies (Makower 1945). The ratio of the heat of sorption to the heat of condensation of water vapour at the same temperature was 2.09 at a moisture content of 0.5%. This ratio was 1.2, with moisture of 5.5% these results indicate the energy necessary to vapourize water. For example, the heat vapourization for eggs containing 0.5% water is more than twice the heat of vapourization of pure water.

In studies on the vapour pressure relationship of dried whole egg containing sucrose and corn syrup solids, it was found that carbondioxide contributed significantly to the total pressure, as measured by a manometric method (Davis and Kline 1965). Although the solution is lower than the vapour pressure of water vapour pressure of pure water, the vapour pressure of liquid egg whites whole eggs, and yolks is substantially the same as that of pure water. This is because vapour-pressure lowering is proportional to the number of molecules of solute in relationship to those of the solvent. Since the molecules in eggs are large, this ratio is very low until the final stages of drying.

TABLE 4

Equilibrium Moisture Content of Egg-Yolk Solids as Related to Relative Humidity and Temperature

Relative humidity (%)	***Equilibrium moisture content (%) at***					
	10°C	***21°C***	***32°C***	***43°C***	***60°C***	***77°C***
10	1.6	1.5	1.4	1.3	1.1	0.8
20	2.5	2.4	2.2	2.0	1.7	1.3
30	3.1	2.9	2.7	2.4	2.1	1.7
40	3.7	3.5	3.3	3.0	2.5	2.0
50	4.3	4.1	3.9	3.6	3.1	2.7
60	5.6	5.0	4.7	4.5	4.1	3.3
70	6.9	6.7	6.6	6.3	5.6	4.4

TABLE-5

Equilibrium moisture Content of Egg-White Solids as Relative Humidity and Temperature

Relative humidity (%)	***Equilibrium moisture content (%) at***					
	10°C	*21°C*	*32°C*	*43°C*	*60°C*	*77°C*
10	5.6	5.4	5.0	4.1	3.7	2.9
20	6.8	6.5	6.0	5.6	4.6	3.5
30	8.4	8.0	7.3	6.6	5.6	4.6
40	10.5	9.9	9.2	8.6	6.9	5.6
50	11.8	11.1	10.6	9.9	8.6	7.4
60	14.6	13.0	12.2	11.8	10.6	8.5
70	18.0	17.6	17.2	16.5	14.4	11.4

2.6 Sand and silica (Bondi, 1989)

Silica, the oxide of silicon, is found in the ash of most plant and animal tissues, in small quantities. The element is believed to function as a biological linking agent, possibly an ether derivative of silica acid of the type R_1–O-Si-O-R_2 and contributes to the structural integrity of connective tissues and bones and of plants as well. Silicon is an integral component of the mucopolysaccharides of cartilage and collagen. In silicon-deficient rats and chicks, bone abnormalities occur because of an impairment of mucopolysaccharide synthesis and formation of cartilage.

In plants, silica serves as a structural component complementing lignin to strength then and rigidify plant cell walls. Silicon dioxide is not digestible and exerts a negative effect upon cell wall digestibility. In some feed stuffs silicon dioxide is found is large amounts as for example, in rice hulls, rice straw and wheat straw. High levels of silicon dioxide in the diet of farm animals may be detrimental. Ruminants grazing in grasses high in silicon dioxide suffer from kidney stones, the main route of excretion of absorbed silicon dioxide being in the urine.

2.7 Total ash characteristics

Many workers observed linear relationship between dietary phosphorus level and tibial ash content. Mikalein and Sell (1982) reported that increased phosphorus level in the diet of layers increased tibial ash content from 57.30 to 59.81 %. This linear relationship between dietary phosphorus level and tibial ash content was also observed by Said *et al* (1984). Bar and Hurwitz (1984), Frost *et al.*, (1990) and Rao *et al.*, (1992).

Contrary to above reports, Mikalein and Sell (1982) reported that dietary phosphorus did not have any significant influence on total ash content of tibial bone. In their experiment, the total ash of tibial bone was 57.30, 59.81 and 57.92 % at 0.26, 0.36 and 0.46 % phosphorus levels respectively. Similar observations were also made by prost *et al* (1990) and Rao *et al* (1992).

The ash content is determined by ignition of a sample at 500°C. Organic compounds are removed at this temperature. The residue represents the inorganic constituents of the sample. Inorganic elements present in organic compounds such as Sulphur and Phosphorus in protein, remain in the ash. The ash may include carbon from organic compounds as carbonate when base-forming minerals are in excess.

The mineral elements occurring in ash have to be determined by special analyses. An ash value higher than normal is generally associated with contamination by sand.

2.8 Egg production and Feed consumption

Many workers observed that egg production was not significantly affected by dietary phosphorus levels in the diet of layers. Hamilton (1980) found no significant difference in egg production (68.90 and 70.20%) between two levels of available phosphorus (0.34 and 0.60%). Similarly, ousterhout (1980) also observed no-significant difference in egg production when the available phosphorus level was decreased from 0.45 to 0.35% in the ration. The egg production was 80.84 and 80.07% at 0.35 and 0.45% available phosphorus levels respectively.

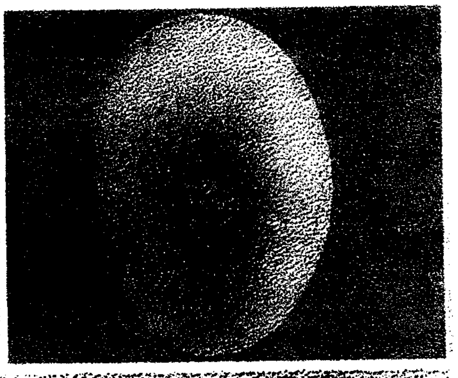
Balnave (1988) indicated that laying hens require approximately 35g calcium per kg diet to maximise the production of eggs with good shell quality. Roland *et al* (1985) found that as the percent dietary calcium was decreased, there was an increase in feed consumption and body weight in layers then increasing dietary calcium level increased the egg production. When feeding trial with limestone as calcium source and found that the feed intake for laying 10 eggs was less by 0.35 percent and when calcium carbonate was fed intake was less by 0.37 percent feed (Georgiev skil *et al.*, 1987). Daily intake was lower for hens fed with diets containing 0.17, 0.23 and 0.30 percent phosphorus and they opined that by adding 10 percent supplemental inorganic phosphorus to the diet hand beneficial effect on feed intake (Miles *et al.*, 1983 and Said *et al.*, 1984). Since it is important to calculate minerals like calcium, phosphorus, Sand and Silica, moisture and total ash in the feeds.

Egg breakage still represents a large economic loss to the poultry industry. It was estimated that 13 to 20% of total eggs produced are cracked or lost before reaching their final destination (Roland, 1988).

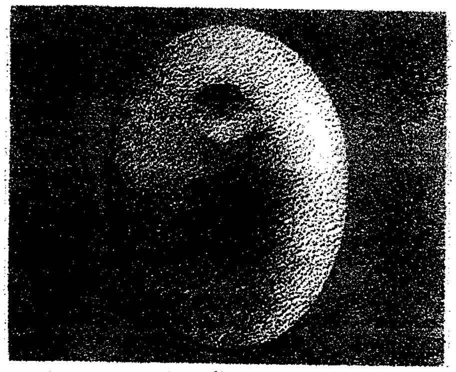
Numerous reports have been presented regarding the effectiveness of feeding various calcium either the pulverized or the granulated form on egg shell quality. Scott *et al* (1971), Roland (1986) and Guinotte (1987) in their reviews have shown a positive effect of calcium with a coarse particle size on egg shell quality in half of the reported studies.

According to Torturo and Centeno (1973), large particle calcium sources increase nitrogen retention and metabolizable energy.

EFFECT OF CALCIUM ON EGG



CALCIUM DEPOSITS
Depósitos de calcio — Dépôt calcaire



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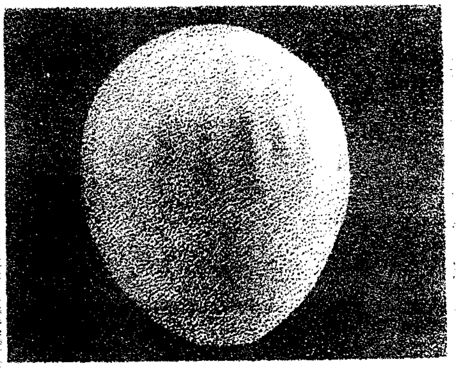

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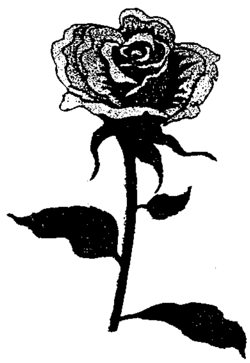
TABLE – 6

Recommended rations (per quintal of feed) for various age groups of Layers and Broilers (Indian poultry Industry year book, 1990)

S.No	Composition	Unit	Chick mash (0-8 weeks of age)	Grower mash (8-20 weeks of age)	Layer Mash		Broiler starter (0-6 weeks of age)	Broiler finisher (6-9 weeks of age)
					Phase I (20-45 weeks)	Phase II (45-80 weeks)		
1.	Yellow maize	kg	29.0	29.0	35.0	40.0	45.0	48.0
2.	Rice Polish	kg	33.7	45.8	32.1	31.1	10.7	17.7
3.	Ground nut cake	kg	22.0	13.0	17.0	12.5	30.1.00	22.0
4.	Lucerne meal	kg	3.0	3.0	3.0	3.0	11.0	1.0
5.	Fish Meal	kg	10.0	7.0	6.0	6.0	40	9.0
6.	DL-Methionine	gm	40	--	--	15	2.0	--
7.	Mineral Mixture	kg	2.0	2.0	3.0	3.0	30.0	2.0
8.	Vitamin $A+B_2+D_3+K$	gm	20.0	20.0	20.0	20.0	20.0	30.0
9.	Vitamin B_{12}	gm	20.0	20.0	20.0	20.0	100.0	20.0
10.	Vitamin K	mg	100.0	100.0	100.0	100.0	20.0	100.0
11.	Potassium iodide	mg	20.0	20.0	200.0	200.0	10.0	20.0
12.	Managanese sulphate	gm	5.0	3.0	3.0	3.0	10.0	8.0
13.	Zinc carbonate	gm	8.0	5.0	3.0	3.0	50.0	8.0
14.	Antribiotic feed supplement	gm	50.0	50	50	50.0	100.0	50.0
15.	Zinc Bacitracin	gm	100.0	100.0	--	--	50.0	100.0



MATERIALS AND METHODS

CHAPTER III

MATERIALS AND METHODS

In this chapter the details of the study area, feed sample collection and methods of analysis are presented.

3.1	Study area
3.2	Reasons for choosing the study
3.3 -3.7	Estimation of Ca, P, SS, M and TA
3.8	Processing of feed sample
3.9	Statistical Tools

3.1 Study area (Namakkal Annual Report, 1998-1999)

Namakkal is geographically situated at 11°N 15 lattitude and 78° E 9 longitude with an altitude of 404 m., MSL and falls under North western agroclimatic zone of Tamil Nadu State.

The average annual rainfall is 900 mm (631-1248) of which 43% is received in North-East monsoon, 34% received in South-West monsoon, 18.38% in summer and 5.4% during the winter. There is no perennial river in Namakkal district except river cauvery, which runs in the border areas only. The major crops grown are sugarcane, tapioca, ground nut and sorghum, under irrigated and rainfed conditions.

3.1.1 Poultry Industry

A majority of farmers are involved in poultry farming (>6000) especially layers. The population of layers is around 10 million in this area which produces 80,00,000 eggs daily. There are about 180 compounded feed manufactures who produce more than 1500 tonnes of feed per day. The annual turnover of poultry industry in Namakkal area is around 400 crores. The eggs are marketed in Kerala and exported to Arabian countries apart from satisfying the needs of this state.

Since this area has been showing a phenomenal growth in the poultry sector. The farmers in this tract adopt scientific methods of housing, feeding and disease control due to which Tamil Nadu stands second, in India, in egg production.

Namakkal is facing a moderately severe summer (March to May) two monsoon periods (South-West Monsoon: June to September) and (North East monsoon: October to December) and winter (January to February). The weather is highly variable, hot in summer and cold in winter, sudden heavy downpour during the two monsoon periods or failure of monsoons to varying extents leading to complications in the poultry as heat stroke, out break of diseases etc.

A sudden increase of 2 to 3° C in the day temperature during summer would result in high mortality of birds and a drop in egg production.

1. An increase in mortality by 0.05%

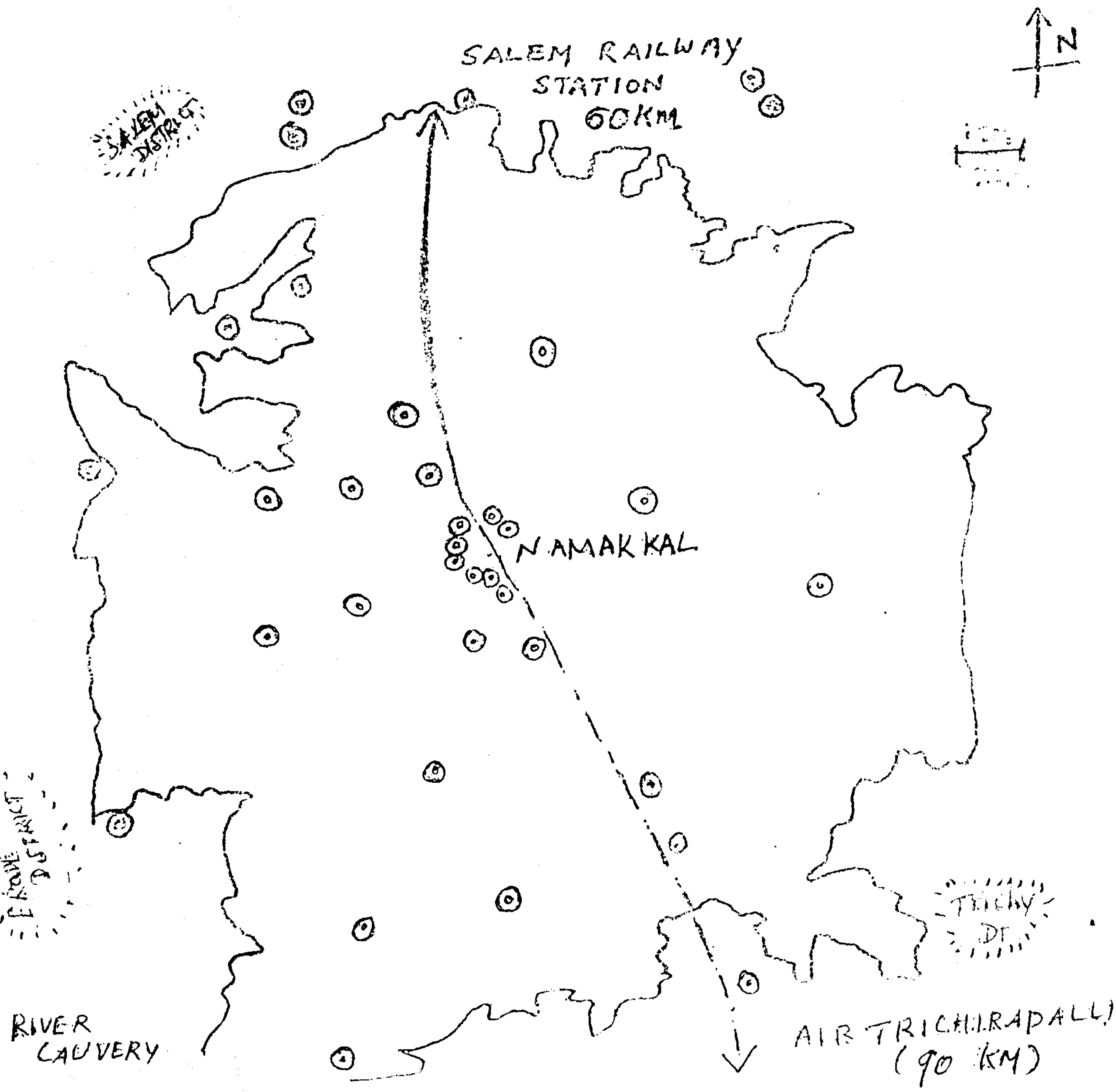
Total layer population	= 100 lakhs
Mortality 0.05%	= 5000 birds
Cost of one bird	= Rs. 100/-
Loss through mortality	= Rs. 5 lakhs/-

2. A drop in egg production to a tune of 8-12 percents.

Egg production per day	= 80 lakhs
10% drop	= 8 lakhs
Cost of one egg	= Rs. 1.30 P
Loss through drop in egg production	= 10.40 lakhs
Total loss per day	= 15.40 lakhs

FIGURE – I

LOCATION OF THE STUDY – NAMAKKAL DISTRICT



3.2 Reasons for choosing the study

To the commercial poultry farmer, hen is essentially an egg/meat producing machine, his objective in feeding his birds is to produce salable eggs/meat of good quality at the lowest possible cost. His economy is determined by the actual cost of the feed that a bird eats, say laying a dozen of eggs or producing a kilogram of meat, and not the price per kilogram of feed.

With the feed costs being the single biggest element in the farm operations, the efficiency with which the feeding is handled will reflect on his profitability. If a bird lays even five more eggs per year with a given feed, then the farmer's profitability increases by 30 percent. Or, if his bird eats only three grams less of feed every day, his profit goes up by 20 percent.

Normally, a hybrid hen eats about 40 kg of feed per year (about 110 g a day) and lays about 20 dozen eggs or more. A broiler chick would consume about three kg of feed in six weeks it matures ready for market. The quantity and quality of eggs / meat and the amount of feed consumed depends on the nutritive value (protein level) and calories (metabolizable energy) of the feed.

Research has indicated that a marked improvement in shell quality can be obtained by feeding part of the dietary calcium as oyster shell or limestone chips. This is especially true if limestone flour rather than a granular source of limestone is used. The hen's requirements for calcium is relatively low, except at the time of the day when egg shell formation is taking place. The intake of oyster shell or calcium at various times during a normal 16 hour day, with the control diet, the hen had no choice as to the time of day calcium was consumed. However, when given the simultaneous choice of diets providing energy, protein or calcium the hen did have a choice as to when eat to calcium. It can be noted that the hen consumed little or no calcium until the afternoon. This is the time of the day that the egg generally would be in the shell gland, hence the requirement for calcium should be higher.

Reviewing the literature, the importance to study the macro and micro nutrients present in different samples collected from different feed processing units is revealed, for which the present study aims at.

3.3 Estimation of calcium

The amounts of calcium present in the feed samples were estimated by potassium permanganate titrations.

Procedure

1) Two grams of feed/ one gram of powder was taken.

2) The feed sample was placed in a silica crucible, heated till smoke ceases, kept it in a muffle furnace at 550° C for an hour and cooled.

3) For calcareous powder sample (Calcite, Shel grit, minigrit, lime stone etc), ashing is not necessary.

4) Then added 1:3 hydrochloric acid till effervescence ceases and then added 1:3 nitric acid, slightly warmed and cooled.

5) Filtered using whatman No-42 filter paper and collected the filtrate in a 100ml standard flask, made the volume upto the mark with distilled water.

6) Took 5 ml aliquot (for calcareous powder samples) /100 ml aliquot (for feed sample) into a conical flask.

7) Added 2 drops of methyl red indicator followed by 1:3 ammonium hydroxide till the solution becomes yellow colour.

8) Added 1:3 hydrochloric acid till the solution becomes pink colour (pH 2.5-3). warmed the solution and measured optical density at 660nm which corresponds to 8μg of standard of phosphorous.

9) Added about 5 ml/10 ml of hot 4.2% ammonium oxalate solution when Ca gets precipitated as calcium oxalate.

10) Heated for five minutes.

11) Filtered through whatmann No-42 filter paper and washed it with (1:50) ammonium hydroxide till ammonium oxalate free from the precipitate checked for excess oxalate with 2.5% barium chloride solution.

12) The precipitate was dissolved in 10 ml of hot (1:4) sulphuric acid .

13) A hole in the filter paper was made and transferred the precipitate in 10 ml of hot sulphuric acid.

14) The solution was warmed to 60° C and titrated with 0.1N potassium permangante solution. The end point is the first appearance of permanent pink colour.

Calculation

For calcareous sample

$$\text{Percentage of calcium} = T.V \times 0.002 \times \frac{100}{5} \times \frac{100}{\text{SampleWeight}}$$

For feed sample

$$\text{Percentage of calcium} = T.V \times 0.002 \times \frac{100}{10} \times \frac{100}{\text{Sample Weight}}$$

T.V - Titre Value

1 ml of 0.1 N potassium = 0.002 g of calcium

3.4 Estimation of phosphorus

The amounts of phosphorus present in the feed samples were estimated by colorimetric method.

Procedure

1) 2 g of the feed sample was taken in a silica crucible. Heated slightly till smoke ceases and kept it in a muffle furnace preheated to 550° C for 2 hours, cooled (for samples other than feed, ashing is not necessary).

2) To this total ash was added (1:3) hydrochloric acid till effervescence ceases, added 5 drops (1:3) nitric acid.

3) Heated and cooled. Filtered through whatmann No 42 filter paper and the filtrate was collected in a 100 ml volumetric flask.

4) The residue was washed with hot water 2 or 3 times and made upto the mark using distilled water (for mineral mix samples dilute 1 ml of this solution water).

5) 0.1 ml of this diluted sample solution (for mineral mix sample) / 0.1 ml aliquot was taken without dilution (for feed sample) in a test tube added 2.9 ml of distilled water, 1 ml of ammonium molybdate and 0.4 ml of 1-amino-2-naphthol-4-sulphonic acid (ANSA) solution.

6) Switched on the colorimeter as soon as 1-amino-2-naphthol-4-sulphonic acid (ANSA) solution was added and time noted for 20 minutes (Blank was prepared by taking 3 ml of distilled water 1 ml of ammonium molybdate and 0.4 ml of ANSA solution).

7) Colorimeter was adjusted for zero reading after 20 minutes with the blank and optical density of the sample solution was measured at 660 nm.

8) The optical density for phosphorus standard solutions of 8 μg and 16 μg was also measured.

9) The optical density of the standard phosphorus nearer to optical density of the sample solution was taken for calculation.

Calculation

For feed sample

$$\left.\begin{array}{l}\text{Percentage of}\\ \text{Phosphorus}\end{array}\right\} = \text{O.D} \times \frac{8}{\text{O.D std}} \times \frac{100}{0.1} \times \frac{100}{\text{Sample Weight}} \times \frac{1}{10^6}$$

For mineral mix sample

$$\left.\begin{array}{l}\text{Percentage of}\\ \text{Phosphorus}\end{array}\right\} = \text{O.D} \times \frac{8}{\text{O.D Std}} \times \frac{100}{0.01} \times \frac{100}{\text{Sample Weight}} \times \frac{1}{10^6}$$

O.D-Optical Density

O.D Std.-Optimal Density Standard

3.5 Estimation of acid insoluble ash (Sand and silica)

Procedure

1) Wet the ash in the silica crucible by adding a few drops of distilled water from the sides. Added about 20 ml of 1:3 hydrochloric acid till effervescence stops (effervescence will be observed only when calcium carbonate is present in the feed stuff).

2) If effervescence was not observed, added about 5 ml / 10 ml 1:3 hydrochloric acid until most of the ash was dissolved. The added about 5 drops of 1:3 nitric acid.

3) The silica crucible was heated to complete the dissolution.

4) Filtered through Whatmann No. 42 into a 100 ml volumetric flask. Wet the residue till it is acid free with hot distilled water.

5) Made the volume upto the mark and kept it for the estimation of minerals.

6) The filter paper containing the insoluble material was placed back into the silica dish and ignited in an electrical bunsen burner at 600°C till smoke free and kept it in the muffle furnace at 600°C for hours.

7) Cooled in a desiccator at room temperature and weighed.

Calculation

$$\left.\begin{array}{l}\text{Percentage of Acid insoluble ash} \\ \text{(Sand and Silica)}\end{array}\right\} = \frac{\text{Weight of insoluble ash x 100}}{\text{Weight of sample}}$$

3.6 Moisture : (Instrinstic moisture or air dried moisture)

Procedure

2 g of the feed sample was taken in a clean dry porcelain crucible and kept it in an air oven at 120° C for 3 hours. The crucible was taken out, cooled it in a desiccator and the loss in weight was found.

Calculation

$$\text{Percentage of moisture} = \frac{\text{Loss in Weight}}{\text{Weight of sample}} \times 100$$

3.7 Total ash

Procedure

1) Weighed accurately 2 g of sample in a silica crucible.

2) The sample was ignited in a burner till smoke ceases off.

3) The crucible was placed in a muffle furnace and heated to 600° C, kept it for 2 hours. At this temperature all organic matter will be burnt off leaving behind minerals.

4) Removed the crucible from the furnace carefully and cooled it in desiccator to room temperature and weighed again.

Calculation

$$\text{Percentage of total ash} = \frac{\text{Weight of the ash}}{\text{Weight of sample}} \times 100$$

The ash may be used for the determination of mineral and acid insoluble ash. Since the ash is hygroscopic weighing should be done quickly.

TABLE-7

Methodology followed parameter wise

Parameters	***Methodology followed***	***Appendix No***
Calcium	$KMnO_4$ method	I
Phosphorus	Colorimetric method	II
Moisture	Air oven method	-
Total ash	Air oven method	IV

3.8 Processing of feed samples

The representative feed sample was spread in this layer at room temperature. The sample was mixed thoroughly and reduced the quantity through quartering method. The sample was pulverized and then collected the material in a stoppered bottle with label and used for laboratory analysis.

3.9 Statistical Tools

3.9.1 Correlation

Simple and multiple correlations were worked out as per the method given by Pearson (1986).

3.9.2 Mean

The means of all the variables were worked out. Difference in means values of calcium, phosphorous, sand and silica, moisture, total ash for different feeds-large sample test.

3.9.3 Regression

Multiple regression was worked out. The analysis was carried out the relationship between dependent and independent variable, when measured simultaneously. Regression equations were formulated using the formula.

$$Y = a + b_1x_1 + b_2x_2 + b_3x_3 + b_4x_4 + b_5x_5$$

Ca - Dependent variable

TA, M, SS and P – Independent variables

a = Constant

b_1 to b_5 = Co-efficients of Ca, P, SS, TA, M

$$b = \frac{\sum xy - \bar{x}\sum y}{\sum x^2 - \bar{x}\sum x}$$

Significance

Significance at 0.01 and 0.05 level of probabilities were arrived at 't' values given in the Table 29.

RESULTS AND DISCUSSION

CHAPTER IV

RESULTS AND DISCUSSION

The results of this study 'Assessment and correlation study of macro and micro nutrients in feeds manufactured in various processing units at Namakkal district', are presented and discussed in the light of objectives set.

Twenty five representative samples each collected from different feed processing units at Namakkal district were analysed for chemical properties of Ca, P, TA, M and SS in different varieties like Layer mash, Grower mash, Chick mash, Broiler starter mash and Broiler finisher mash. The availability of Ca, P, TA, M, SS were estimated with the help of potassium permanganate method, Colorimetric method, Air oven method, Air oven method respectively.

The sample wise analytical data are represented in Table (8-12) for LM, GM, CM, BSM and BFM in Namakkal district.

The availability of nutrients are furnished in Table (13) with maximum, minimum and mean values in systematic manner.

4.1. Maximum, Minimum and mean values of M, Ca, P, SS, TA in LM, GM, CM, BSM and BFM

4.1.1 Moisture

The moisture ranged from 7.52 to 11.83 percent with the mean value of 10.056 percent in the Layer mash, 8.16 to 11.81 percent with the mean value of 9.656 percent in the Grower mash, 6.78 to 11.89 percent with the mean value of 9.6476 percent in the chick mash, 8.8 to 12.38 percent with the mean value of 9.8792 percent in the Broiler starter mash and 8.63 to 11.97 percent the mean value of 10.0424 percent in the Broiler finisher mash of Namakkal district.

Feeds were manufactured in different processing units, the moisture content of the feed was less than the ISI requirement in all the varieties.

4.1.2 Calcium

The feed reaction Ca had a variation from 3.09 to 5.34 percent with a mean value of 4.0356 percent in Layer mash 1.2 to 2.84 percent with a mean value of 1.6668 percent in Grower mash, 1.15 to 2.2 percent with a mean value of 1.5688 percent in Chick mash, 0.55 to 2.39 percent with a mean value of 1.5052 percent in Broiler starter mash and 0.95 to 2.1 percent with a mean value of Broiler finisher mash in Namakkal feeds.

4.1.3 Phosphorus

The phosphorus content varies from 0.29 to 1.12 percent in Layer mash, 0.63 to 1.31 percent in Grower mash, 0.37 to 1.22 percent in chick mash, 0.33 to 0.89 percent in Broiler starter mash and 0.36 to 1.14 percent in Broiler finisher mash with a mean value of 0.7376, 0.8316, 0.8068, 0.696 and 0.6288 percent respectively. The amount of P was greater than the required values, was accounted from maximum, minimum and mean values.

4.1.4 Sand and Silica

The data reported that Sand and Silica content varied from 0.55 to 5.89 percent in Layer mash 1.04 to 5.26 percent in Grower mash, 0.95 to 7.24 percent in Chick mash, 0.39 to 5.09 percent in Broiler starter mash and 0.44 to 3.9 percent in Broiler finisher mash with a mean values of 2.58, 1.91, 2.5956, 1.47 and 1.5244 percent respectively.

SS values in different feeds and different varieties reflected the low values of SS in comparison with standard values.

TABLE-8

Sample wise analytical data (Layer Mash)

S.No	M	Ca	P	SS	TA
1	11.21	3.09	0.29	1.77	9.05
2	10.38	4.39	0.8	1.06	9.56
3	11.39	4	0.66	0.55	9.19
4	8.64	4.5	0.54	1.33	9.11
5	9.77	3.6	0.62	2.29	11.29
6	11.49	4.39	0.74	1.72	11.26
7	9.48	4.19	0.72	1.5	10.26
8	11.77	3.89	0.5	2.38	10.76
9	9.8	3.84	0.82	1.33	13.72
10	9.53	4.39	0.73	5.21	19.33
11	11.83	4.03	0.79	2.49	13.33
12	11.26	3.8	1.12	2.67	14.6
13	11.63	4.49	0.84	2.42	16.11
14	10.8	3.79	0.85	2.1	12.17
15	9.97	3.44	0.86	5.89	15.75
16	9.22	5.34	0.79	4.1	19.5
17	9.3	3.69	0.76	2.9	11.83
18	9.98	3.44	0.9	2.96	15.41
19	9.52	3.95	0.71	2.58	15.01
20	10.7	4.04	0.73	2.05	14.92
21	8.63	4.39	0.63	2.81	13.36
22	9.2	3.54	0.87	3.48	15.05
23	10.65	4.39	0.73	3.29	17.25
24	8.53	4.09	0.83	2.39	12.34
25	9.02	4.19	0.61	1.68	13.68

TABLE – 9

Sample wise analytical data (Grower Mash)

S.No	M	Ca	P	SS	TA
1	11.71	1.59	0.83	1.89	7.57
2	11.8	1.25	1.31	2.27	9.22
3	10.67	1.25	1.03	1.84	7.63
4	9.14	1.6	1.16	3.59	11.38
5	8.16	1.65	0.74	5.26	11.05
6	8.41	2.84	0.74	1.27	9.02
7	8.62	2.22	0.77	1.16	8.41
8	8.67	1.54	0.89	1.08	7.55
9	8.94	1.2	0.9	1.28	6.99
10	9.7	1.85	0.66	2.66	8.66
11	8.68	1.64	0.7	1.16	6.56
12	8.43	1.24	0.83	1.68	7.02
13	8.86	1.89	0.76	2.09	6.38
14	9.61	1.84	0.97	3.19	9.42
15	10.31	1.65	0.63	1.83	7.61
16	10.43	1.87	0.67	2.1	9.22
17	9.75	1.73	0.78	1.81	8.47
18	11.62	1.56	0.73	2.2	9.12
19	9.67	1.72	0.76	1.28	8.56
20	8.62	1.63	0.81	1.27	6.76
21	8.71	1.24	0.91	1.04	7.27
22	9.64	1.53	0.65	1.32	8.23
23	10.12	2.01	0.7	1.16	7.21
24	11.81	1.72	1.03	1.62	6.52
25	9.32	1.41	0.83	1.7	6.35

TABLE – 10

Sample wise analytical data (Chick Mash)

S.No	M	Ca	P	SS	TA
1	11.31	1.4	0.37	2.59	8.54
2	11.89	1.8	0.75	2.19	8.08
3	9.48	2.2	0.82	2.39	7.18
4	11.24	1.6	1.22	1.87	8.96
5	11	1.3	0.94	2.16	8.11
6	9.86	1.3	0.81	1.67	7.7
7	6.78	1.4	0.65	4.48	9.85
8	9.16	1.15	0.55	2.9	5.36
9	10.46	1.45	0.99	2.27	8.68
10	8.87	1.94	0.73	1.18	7.44
11	8.39	1.25	0.86	1.54	6.64
12	9.6	1.45	0.77	7.24	12.44
13	9.85	1.89	0.96	0.95	4.66
14	9.34	1.79	0.76	3.05	9.58
15	8.99	1.99	0.87	3.25	10.91
16	8.78	1.92	0.72	2.67	8.55
17	8.53	1.31	0.78	2.27	8.12
18	8.71	1.48	0.67	2.42	7.23
19	9.26	1.51	0.98	1.93	8.92
20	9.45	1.45	0.66	2.19	8.17
21	8.77	1.16	0.82	1.65	7.83
22	10.51	1.83	0.93	3.48	9.35
23	11.32	1.8	0.75	2.96	6.47
24	11.21	1.27	0.97	2.47	8.72
25	8.43	1.58	0.84	3.12	7.54

TABLE – 11

Sample wise analytical data (Broiler Starter Mash)

S.No	M	Ca	P	SS	TA
1	9.83	1.5	0.66	1.63	6.36
2	9.97	1.99	0.78	1.59	7.66
3	10.67	1	0.6	1.64	5.54
4	12.02	1.19	0.33	1.19	7.66
5	12.38	1.4	0.64	3.83	5.51
6	8.94	1.3	0.61	0.9	6.77
7	9.96	1.35	0.6	1.57	7.05
8	9.02	1.3	0.68	0.59	6.37
9	9.25	0.95	0.64	1.12	5.6
10	8.92	0.55	0.47	1.38	5.47
11	8.98	1.6	0.89	0.62	6.86
12	9.59	1.1	0.66	1.08	5.83
13	11.13	1.7	0.76	1.64	8.64
14	11.48	1.5	0.58	1.97	7.46
15	8.93	1.2	0.65	1.62	7.1
16	9.99	1.29	0.6	5.09	8.79
17	9.06	1.24	0.79	2.62	8.1
18	11.21	1.05	0.75	0.89	5.67
19	9.88	1.55	0.88	0.85	3.81
20	8.9	1.79	0.89	0.65	5.7
21	10.9	1.8	0.86	1.45	6.94
22	8.8	2.39	0.8	0.96	6.66
23	8.89	2.29	0.74	0.94	6.71
24	9.28	2.29	0.72	0.39	5.34
25	9	2.31	0.82	0.54	5.78

TABLE – 12

Sample wise analytical data (Broiler Finisher Mash)

S.No	M	Ca	P	SS	TA
1	10.53	1.4	0.63	1.5	5.99
2	8.93	1.39	0.53	2.54	5.16
3	11.43	1.49	0.52	1.71	6.14
4	11.97	1.5	0.5	1.87	6.87
5	11.23	1.2	0.36	0.44	6.28
6	10.55	1.2	0.65	3.9	5.8
7	9.56	1.25	0.59	2.27	7.53
8	10.36	1.25	0.69	1.62	6.92
9	8.74	1.4	0.69	0.74	5.62
10	9.58	1.15	0.6	0.82	5.34
11	9.21	1.15	0.62	1.27	6.03
12	9.36	1.25	0.65	1.72	6.19
13	10.6	1.35	0.51	1	5.34
14	11.13	1.3	1.14	1.06	5.09
15	10.99	0.95	0.41	1.03	5.1
16	9.96	1.35	0.59	1.36	6.75
17	10.62	1.2	0.54	2.13	6.58
18	9.42	1.2	0.41	1.86	7.26
19	9.56	1.15	0.68	1.52	6.91
20	8.63	1.34	0.75	2.12	6.67
21	10.69	1.45	0.75	0.97	5.44
22	9.67	1.1	0.76	1.02	3.96
23	9.35	1.99	0.83	1.65	6.68
24	9.63	2.1	0.69	1.43	4.88
25	9.36	1.9	0.63	0.56	5.28

TABLE – 13

Maximum, Minimum and Mean values of M, Ca, P, SS, TA in Layer mash, Grower mash and Chick mash

Parameters	*Layer Mash*			*Grower Mash*			*Chick Mash*		
	Maximum	*Minimum*	*Mean*	*Maximum*	*Minimum*	*Mean*	*Maximum*	*Minimum*	*Mean*
M	11.83	7.52	10.056	11.81	8.16	9.656	11.89	6.78	9.6476
Ca	5.34	3.09	4.0356	2.84	1.2	1.6668	2.2	1.15	1.5688
P	1.12	0.29	0.7376	1.31	0.63	0.8316	1.22	0.37	0.8068
SS	5.89	0.55	2.518	5.26	1.04	1.91	7.24	0.95	2.5956
TA	19.5	9.05	13.3608	11.38	6.35	8.0872	12.44	4.66	8.0212

Maximum, Minimum and Mean values of M, Ca, P, SS, TA in Broiler Starter mash and Broiler Finisher mash

Parameters	*Broiler Starter Mash*			*Broiler Finisher Mash*		
	Maximum	*Minimum*	*Mean*	*Maximum*	*Minimum*	*Mean*
M	12.38	8.8	9.8792	11.97	8.63	10.0424
Ca	2.39	0.55	1.5052	2.1	0.95	1.3604
P	0.89	0.33	0.696	1.14	0.36	0.6288
SS	5.09	0.39	1.47	3.9	0.44	1.5244
TA	8.79	3.81	6.5352	7.53	3.96	5.9924

4.1.5 Total ash

The total ash content indicated a range of 9.05 to 19.5 percent with a mean of 13.3608 percent, 6.35 to 11.38 percent with a mean of 8.0872 percent, 4.66 to 12.44 percent with a mean of 8.0212 percent 3.81 to 8.79 percent with a mean value of 6.5352 percent and 3.96 to 7.53 percent with a mean of 5.9924 percent in Layer mash, Grower mash, Chick mash, Broiler starter mash and Broiler finisher mash respectively. The low contents of TA in various feeds were indicated in the table.

In various feeds the moisture content and Sand and Silica are less than the standard values. The amount of Ca and P present are lightly greater than the ISI requirement in Layer mash, Grower mash, Chick mash, Broiler starter mash and Broiler finisher mash.

Our results indicated that the feed processing units concentrated on the major nutrients such as Ca and P than minor nutrients such as Sand and Silica.

Belyavin and Marangon (1987) also reported than hens have the ability to extra Ca and P in the skeleton to argument dietary supply during shell formation.

This result highlighted the report presented by outsterhout (1980), Roland (1994), Abdullah *et al.* (1993). The high dietary Ca was essential for the increase in egg specific gravity.

The high content of P was importance for the increase in egg production. This findings was on par with the studies of Hess and Briton (1997).

4.2 Correlation studies

By applying the principle of simple correlation and multiple regression analysis the interrelationships of the properties of feeds were carried out.

TABLE - 14

Inter correlation between variables in Layer mash

Variables	*Ca*	*M*	*P*	*TA*	*SS*
Ca	1.000				
M	-0.177	1.000			
P	0.077	0.013	1.000		
TA	0.001	-0.209	0.294	1.000	
SS	0.322	-0.142	0.466*	0.747**	1.000

* Correlation is significant at the 0.05 level
** Correlation is significant at the 0.01 level.

On the impact of Ca on moisture, Ca accounted for a negative association with all the variables. A minimum positive association of Ca with SS was observed (r = 0.322). The tabulation accounted a positive association of Ca with P (r = 0.070) and a minimum positive association was observed by Ca with TA (r = 0.001). M accounted for a negative association with TA. A maximum positive association of M with P was observed (r = 0.013). Similarly positive association were exhibited by P with TA and SS (r = 0.466**). Correlation co-efficient values accounted higher association of TA with SS while others and strengths of associations were depicted in Table 14.

TABLE - 15

Inter correlation between variables in Grower mash

Variables	*Ca*	*M*	*P*	*TA*	*SS*
Ca	1.000				
M	-0.192	1.000			
P	-0.406*	0.284	1.000		
TA	-0.038	-0.007	0.174	1.000	
SS	0.209	0.008	0.178	0.732**	1.000

* Correlation is significant at the 0.05 level
** Correlation is significant at the 0.01 level.

On the impact of Ca on moisture, Ca accounted for a negative association. A positive association of Ca with SS was observed (r = 0.209). A maximum negative association of Ca with P (r = 0.406*). The co-efficient furnished in Table 15 a maximum positive association of M with P (r = 0.284) and P with TA and SS. The co-efficient values enlisted a negative association with M with TA and P with Ca (r = 0.406*). A positive association of TA with SS was observed (r = 0.732**). The other variables reflected a mild positive association.

TABLE - 16

Inter correlation between variables in Chick mash

Variables	*Ca*	*M*	*P*	*TA*	*SS*
Ca	1.000				
M	0.094	1.000			
P	0.123	0.229	1.000		
TA	-0.026	-0.144	-0.218	1.000	
SS	0.036	-0.034	0.096	0.691**	1.000

** Correlation is significant at the 0.01 level.

On the impact of Ca on M, Ca accounted a positive association. A maximum positive association of Ca with SS was observed (r = 0.123). The tabulation accounted a negative association of Ca with TA. A minimum positive association was observed by Ca with SS. The maximum positive association of TA with SS was accounted which is indicated by the correlation co-efficient values (r = 0.691**). Further results indicated a maximum positive association of P with M. The negative association of P with TA. The other variables accounted for mild association.

TABLE - 17

Inter correlation between variables in Broiler starter mash

Variables	*Ca*	*M*	*P*	*TA*	*SS*
Ca	1.000				
M	-0.196	1.000			
P	0.578**	- 0.343	1.000		
TA	- 0.260	0.396	-0.271	1.000	
SS	0.041	0.198	-0.271	0.496*	1.000

* Correlation is significant at the 0.05 level

** Correlation is significant at the 0.01 level.

On the impact of Ca on M, Ca accounted a negative association. A maximum positive association of calcium with phosphorus (r = 0.578**) and minimum association of Ca with SS, Ca with TA was negatively correlated. The correlation Table 17 enlisted that M showed negative association with P. A maximum positive association of M with TA and a minimum association of M with SS. The tabulation indicated a negative association of P with TA and SS. TA on SS, TA accounted for a maximum positive association with SS (r = 0.496*).

TABLE -18

Inter correlation between variables in Broiler finisher mash

Variables	*Ca*	*M*	*P*	*TA*	*SS*
Ca	1.000				
M	-0.148	1.000			
P	0.248	- 0.174	1.000		
TA	- 0.062	- 0.126	-0.071	1.000	
SS	- 0.080	0.006	-0.249	0.221	1.000

On the impact of Ca on M, Ca accounted a negative association. A maximum positive association of Ca with Phosphorus, was observed (r = 0.248). The tabulation accounted a negative association of Ca with TA and SS. M accounted a negative association with P and TA a positive association with SS (r = 0.006) was observed. The tabulation accounted a negative association of P with TA and SS. The co-efficient furnished in Table 18, TA accounted for a positive association.

TABLE - 19

Inter correlation of moisture in various feed samples

Variables	*LM*	*GM*	*CM*	*BSM*	*BFM*
LM	1.000				
GM	0.157	1.000			
CM	-0.028	0.343	1.000		
BSM	-0.114	0.035	-0.156	1.000	
BFM	0.590**	0.088	-0.076	-0.010	1.000

** Correlation is significant at 0.01 level.

TABLE - 20

Strength and rank of association of variables for moisture content

Variables	*LM*	*GM*	*CM*	*BSM*	*BFM*
LM	-	+ve	-Ve	-Ve	+Ve
GM	+Ve	-	+Ve	+Ve	+Ve
CM	-Ve	+Ve	-	-Ve	-Ve
BSM	- Ve	+Ve	-Ve	-	-Ve
BFM	+Ve	+Ve	-Ve	-Ve	-

Moisture content of Layer mash showed a positive association with the moisture content of Grower mash and Broiler finisher mash (r = 0.590**) and a negative association with Chick mash and Broiler starter mash. Grower mash showed a positive association with the moisture content of all the various feeds Chick mash showed a negative association with Broiler starter mash and Broiler finisher mash. Moisture content of Broiler starter mash showed a negative association with Broiler finisher mash.

TABLE - 21

Inter correlation of Calcium in various feed samples

Variables	*LM*	*GM*	*CM*	*BSM*	*BFM*
LM	1.000				
GM	0.270	1.000			
CM	0.221	-0.060	1.000		
BSM	0.067	-0.032	-0.089	1.000	
BFM	0.299	-0.091	-0.032	0.556**	1.000

** Correlation is significant at the 0.01 level.

TABLE -22

Strength and rank of association of variables for calcium content

Variables	*LM*	*GM*	*CM*	*BSM*	*BFM*
LM	-	+Ve	+Ve	+Ve	+Ve
GM	+Ve	-	-Ve	-Ve	-Ve
CM	+Ve	-Ve	-	-Ve	-Ve
BSM	+ Ve	-Ve	-Ve	-	+Ve
BFM	+Ve	-Ve	-Ve	+Ve	-

Calcium content of Layer mash showed a positive association with the Calcium content of all the feeds like Grower mash, Chick mash Broiler starter mash and Broiler finisher mash. The tabulation showed a negative positive association of Grower mash with Calcium content of Chick mash Broiler starter mash and Broiler finisher mash. Calcium content of Chick mash showed a negative associative with Broiler starter mash and Broiler finisher mash. Calcium content of Broiler starter mash showed a positive association with Broiler finisher mash (r = 0.556**). While others showed a positive association and strength of association were depicted in Table 22.

TABLE - 23

Inter correlation of phosphorus in various feed samples

Variables	*LM*	*GM*	*CM*	*BSM*	*BFM*
LM	1.000				
GM	-0.169	1.000			
CM	0.276	0.182	1.000		
BSM	0.149	-0.192	-0.142	1.000	
BFM	0.072	0.079	-0.151	0.137	1.000

TABLE - 24

Strength and rank of association of variables for phosphorus content

Variables	*LM*	*GM*	*CM*	*BSM*	*BFM*
LM	-	-Ve	+Ve	+Ve	+Ve
GM	-Ve	-	+Ve	-Ve	+Ve
CM	+Ve	+Ve	-	-Ve	-Ve
BSM	+ Ve	-Ve	-Ve	-	+Ve
BFM	+Ve	+Ve	-Ve	+Ve	-

Phosphorus content of Layer mash was positively associated with phosphorus content of Chick mash, Broiler starter mash and broiler finisher mash and negatively associated with Grower mash. Phosphorus content of Grower mash was positively associated with phosphorus content of Chick mash and Broiler finisher mash and negatively associated with Layer mash and Broiler starter mash. Phosphorus content of Chick mash showed a negatively association with Broiler starter mash and

Broiler finisher mash. Phosphorus content of Broiler starter mash showed a positively association Broiler finisher mash.

TABLE - 25

Inter correlation of phosphorus in various feed samples

Variables	*LM*	*GM*	*CM*	*BSM*	*BFM*
LM	1.000				
GM	-0.050	1.000			
CM	0.020	-0.082	1.000		
BSM	0.207	0.533**	-0.043	1.000	
BFM	-0.323	-0.241	0.045	-0.170	1.000

** Correlation is significant at the 0.01 level.

TABLE - 26

Strength and rank of association of variables for sand and silica content

Variables	*LM*	*GM*	*CM*	*BSM*	*BFM*
LM	-	-Ve	+Ve	+Ve	-Ve
GM	-Ve	-	-Ve	+Ve	-Ve
CM	+Ve	-Ve	-	-Ve	+Ve
BSM	+ Ve	+Ve	-Ve	-	-Ve
BFM	-Ve	-Ve	+Ve	-Ve	-

Sand and Silica content of Lower mash showed a positive association with the Sand and silica content of Chick mash and Broiler starter mash and a negative association with Grower mash and Broiler finisher mash. Grower mash showed a positive association with Sand and Silica content of Broiler starter mash and a negative association with Layer mash, Chick mash and Broiler finisher mash. Chick mash showed a positive association with Layer mash and Broiler finisher mash and a negative association with Grower mash and Broiler starter mash. Broiler starter mash showed a maximum positive association with Grower mash (r = 0.533**) and the Layer mash and a negative association with Layer mash and Broiler finisher mash.

TABLE - 27

Inter correlation of Total ash in various feed samples

Variables	*LM*	*GM*	*CM*	*BSM*	*BFM*
LM	1.000				
GM	-0.211	1.000			
CM	-0.041	0.182	1.000		
BSM	-0.036	0.157	-0.127	1.000	
BFM	-0.064	0.254	-0.103	-0.067	1.000

TABLE - 28

Strength and rank of association of variables for total ash content

Variables	*LM*	*GM*	*CM*	*BSM*	*BFM*
LM	-	-Ve	-Ve	-Ve	-Ve
GM	-Ve	-	+Ve	+Ve	+Ve
CM	-Ve	+Ve	-	-Ve	-Ve
BSM	- Ve	+Ve	-Ve	-	-Ve
BFM	-Ve	+Ve	-Ve	-Ve	-

Total ash content of Layer mash showed a negative association with the total ash content of Grower mash, Chick mash, Broiler starter mash and Broiler finisher mash. Grower mash showed a positive association with the total ash content of Chick mash, Broiler starter mash and Broiler finisher mash and a negative association with Layer mash. Chick mash showed a positive association with total ash content of Grower mash and a negative association with Layer mash, Broiler starter mash and Broiler finisher mash. Broiler starter mash and Broiler finisher mash showed a positive association with Grower mash and a negative association with Layer mash, Chick mash and Broiler starter mash.

The correlation diagram of calcium and phosphorus content in LM, GM, CM, BSM and BFM are depicted in figure 1 and 2.

FIGURE – 2 : CORRELATION DIAGRAM OF Ca PRESENT IN LM, GM, CM, BSM AND BFM

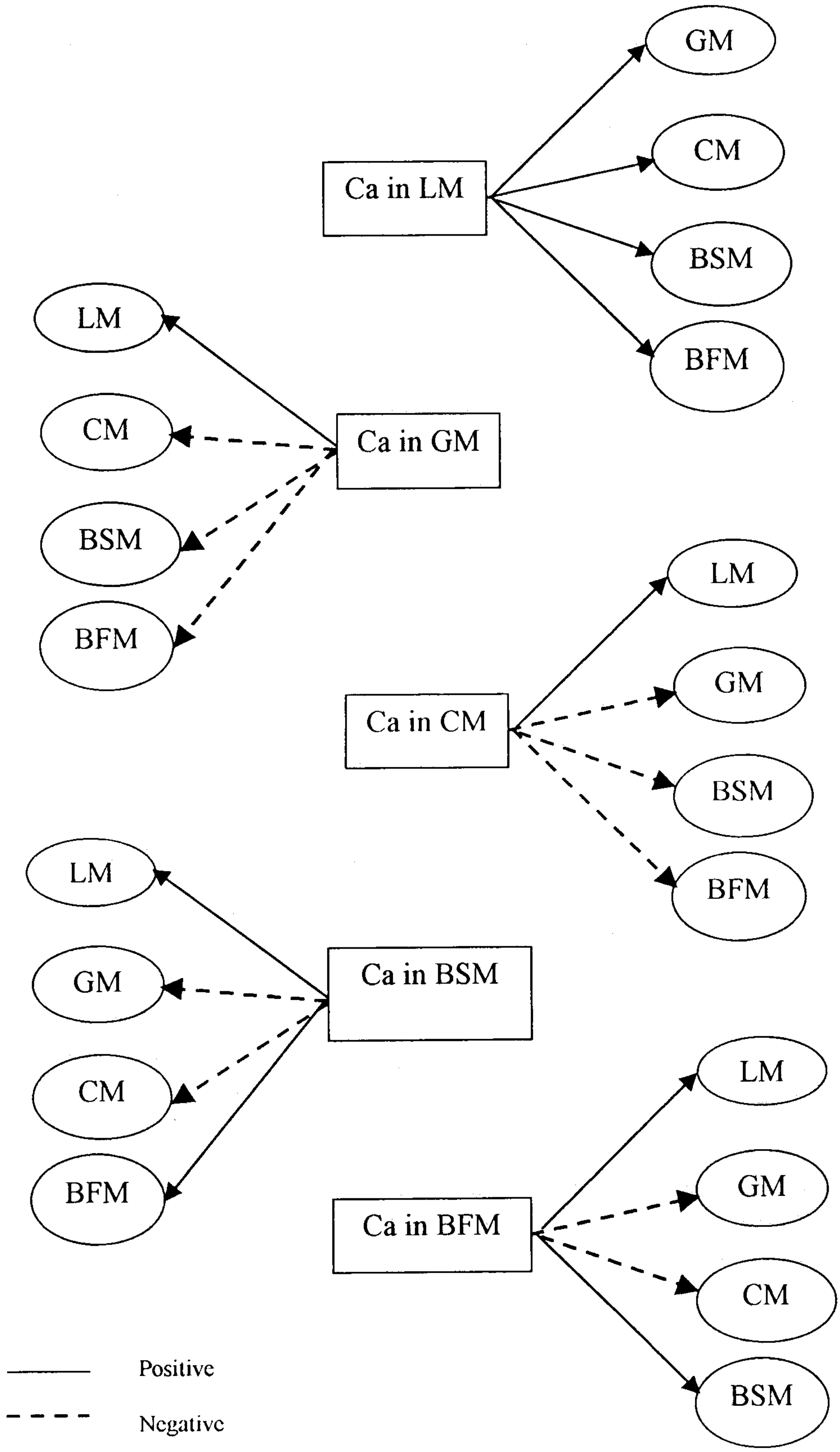


FIGURE – 3 : CORRELATION DIAGRAM OF PHOSPHORUS PRESENT IN LM, GM, CM, BSM AND BFM

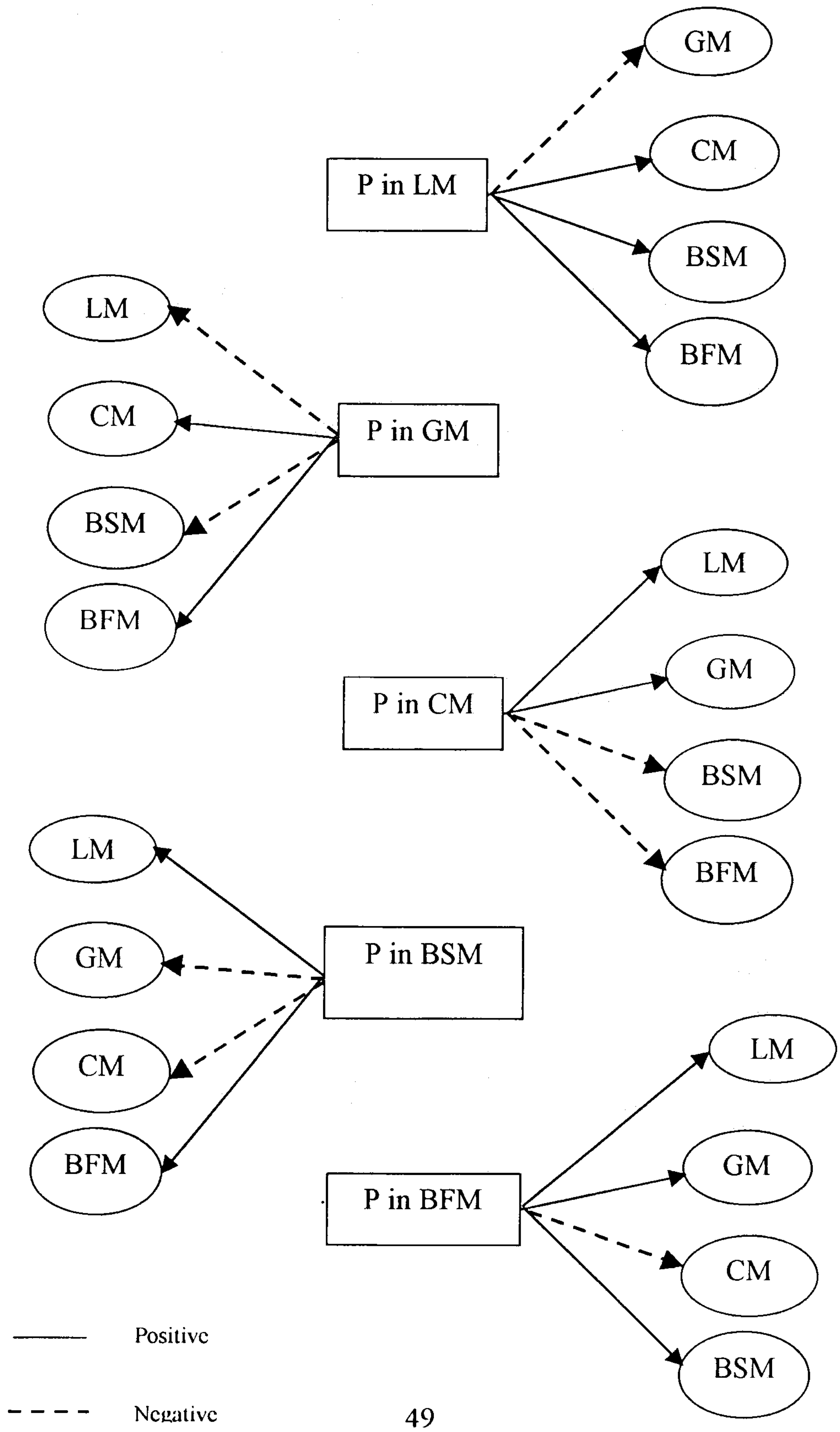


TABLE – 29
MULTIPLE REGRESSION ANALYSIS

S.No	*Variables*	*Layer mash*				*Grower mash*			
		Standardized co-efficient	*Unstandardized co-efficient*		*t-values*	*Standardized co-efficient*	*Unstandardized co-efficient*		*t-values*
		Beeta	*B*	*S.E*		*Beeta*	*B*	*S.E*	
Constant	*Ca*		*4.016*	*1.012*	*3.969*		*1.742*	*0.723*	*2.409*
1	M	-0.180	-7.9	0.085	-0.924	-0.082	-2.50	0.060	-0.425
2	P	-0.125	-0.365	0.634	-0.576	-0.417	-0.894	0.422	-2.117
3	SS	-0.608	-0.232	0.112	-2.078	-0.374	-0.139	0.101	-1.377
4	TA	0.808	0.125	0.049	2.579*	0.554	0.146	0.071	2.052*

S.No	*Variables*	*Chick mash*				*Broiler starter mash*			
		Standardized co-efficient	*Unstandardized co-efficient*		*t-values*	*Standardized co-efficient*	*Unstandardized co-efficient*		*t-values*
		Beeta	*B*	*S.E*		*Beeta*	*B*	*S.E*	
Constant	*Ca*		1.220	0.640	1.907		-0.931	1.166	-0.799
1	M	0.070	1.66	0.055	0.305	0.060	2.63	0.084	0.314
2	P	0.096	0.167	0.427	0.390	0.612	2.161	0.652	3.315**
3	SS	-0.028	-6.6	0.079	-0.084	-0.285	-0.129	0.094	-1.372
4	TA	0.048	8.68	0.59	0.148	0.337	0.133	0.078	1.707

S.No	*Variables*	*Broiler finisher mash*			
		Standardized co-efficient	*Unstandardized co-efficient*		*t-values*
		Beeta	*B*	*S.E*	
Constant	*Ca*		1.538	0.924	1.664
1	M	-0.117	-3.60	0.68	-0.533
2	P	0.220	0.383	0.392	0.976
3	SS	-0.059	-1.9	0.70	-0.264
4	TA	-0.012	-3.8	0.72	-0.053

4.3 Regression analysis

The correlation studies reveal only an association. It measures only the interdependence / intensity of relationship and does not give any idea (prediction) of how far one is directly dependent on the other variable. The regression analysis measures the rate of change of Y.

The multiple regression analysis data with beta, co-efficient, standard error, 't' values and 'B' values for dependent variable for Layer mash, Grower mash, Chick mash, Broiler starter mash and Broiler finisher mash.

Regression estimates are not significantly exhibited. TA exerted positive influence while M, P and SS showed a negative influence on Ca in the feed of Layer mash.

In Grower mash, TA depicted a positive influence while others revealed a negative influence.

A positive influence of M, P and TA with Ca and a negative influence of SS with Ca was claimed from the Chick mash.

In Broiler starter mash M, P and TA depicted a positive influence while SS revealed a negative influence.

P exerted a positive influence while M, TA and SS showed a negative influence on Ca in the feed of Broiler finisher mash.

In all 5 categories, SS showed a negative influence on Ca. An impressive positive significant influence was encountered by Ca with P in Broiler finisher mash and Ca influenced significantly with TA in Grower mash.

4.4 *ISI requirements of Ca, P, M and SS*

The Table 30 shows the ISI measurements of calcium, phosphorus, sand and silica and moisture in feeds and the graph drawn for ISI values of calcium, phosphorus, sand and silica and moisture in Layer mash, Grower mash, Chick mash, Broiler starter mash and Broiler finisher mash.

TABLE-30

ISI requirements for chicken feeds (Indian poultry industry year book, 1997)

S. No	*Characteristic*	*Chick Mash (0-8 weeks)*	*Grower Mash (8-20 weeks)*	*Layer Mash (20-80 weeks)*	*Broiler Starter Mash (0-6 weeks)*	*Broiler Finisher Mash (6-9 weeks)*
1	Moisture, % by Wt. (max)	11	11	11	11	11
2	Acid insoluble ash, % by Wt. (max)	4.0	4.0	4.0	3.0	3.0
3	Calcium, % by Wt. (min)	1.5	1.5	3.75	1.2	1.2
4	Total phosphorus, % by Wt. (min)	0.75	0.75	0.75	0.75	0.75

FIGURE - 4

ISI requirements of Moisture, Sand & Silica, Calcium and Phosphorus in CM, GM, LM, BSM and BFM

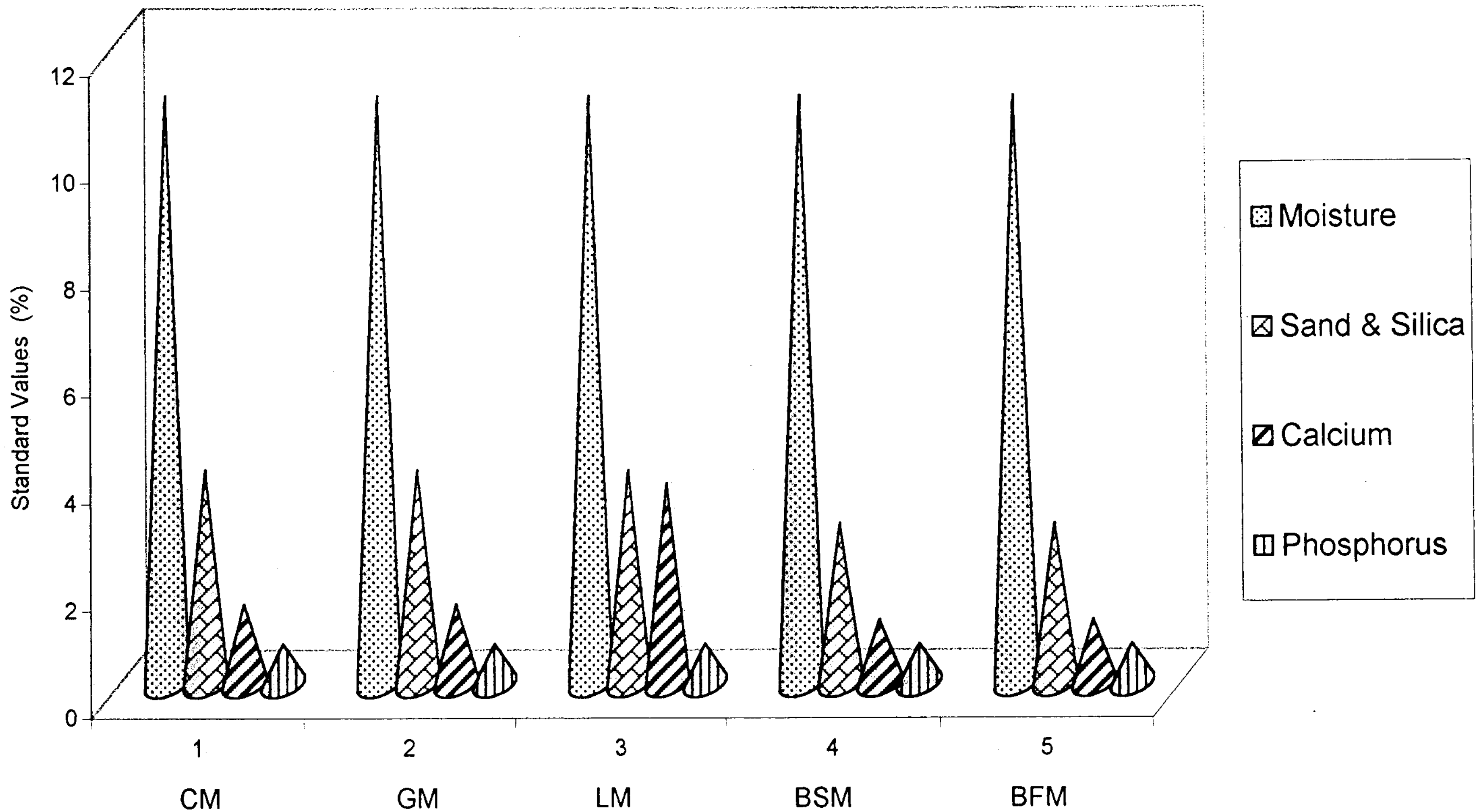


FIGURE - 5

CORRELATION OF Ca WITH P, M, SS AND TA IN LAYER MASH(LM)

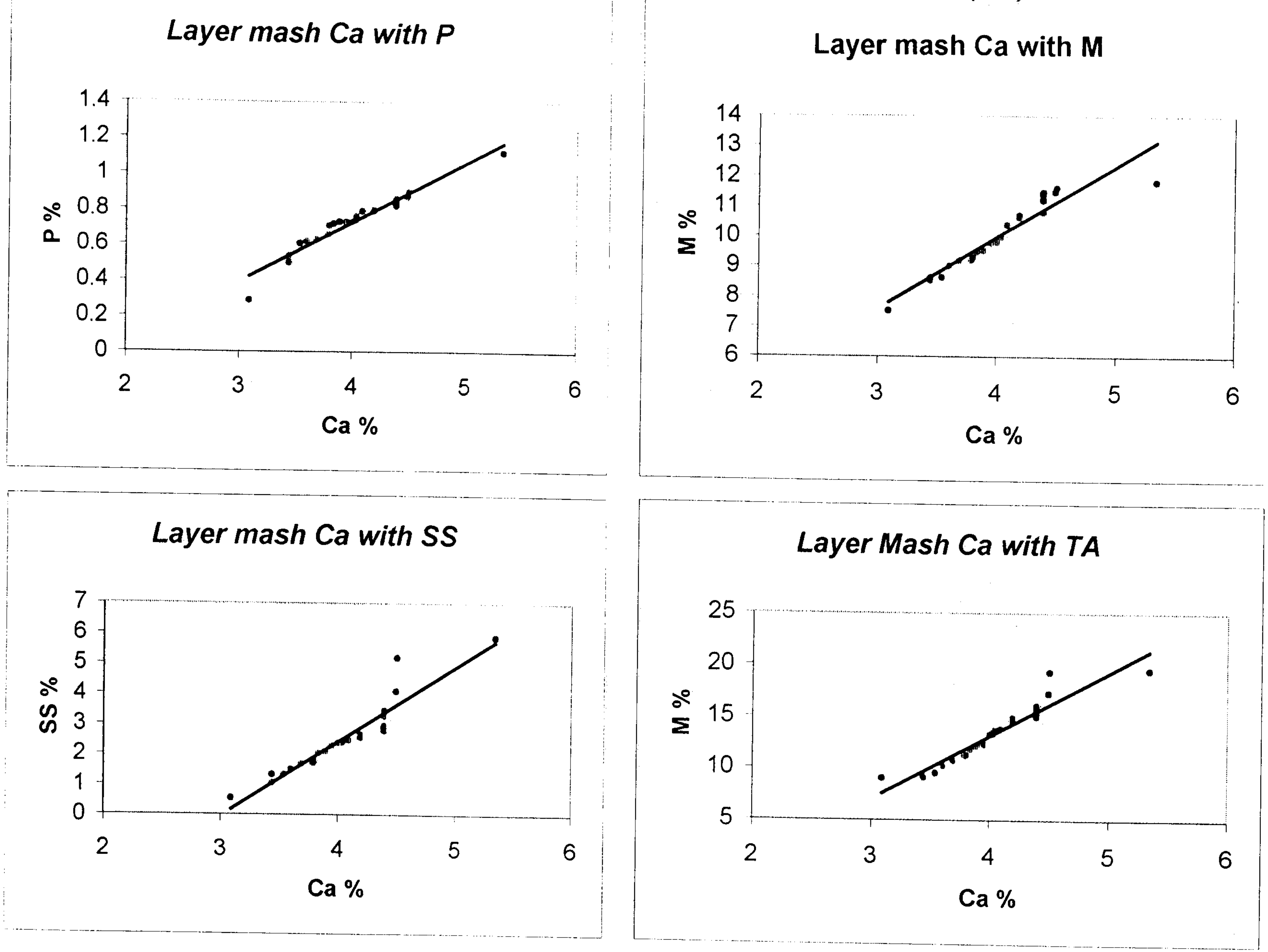


FIGURE - 6

CORRELATION OF Ca WITH P, M, SS AND TA IN GROWER MASH (GM)

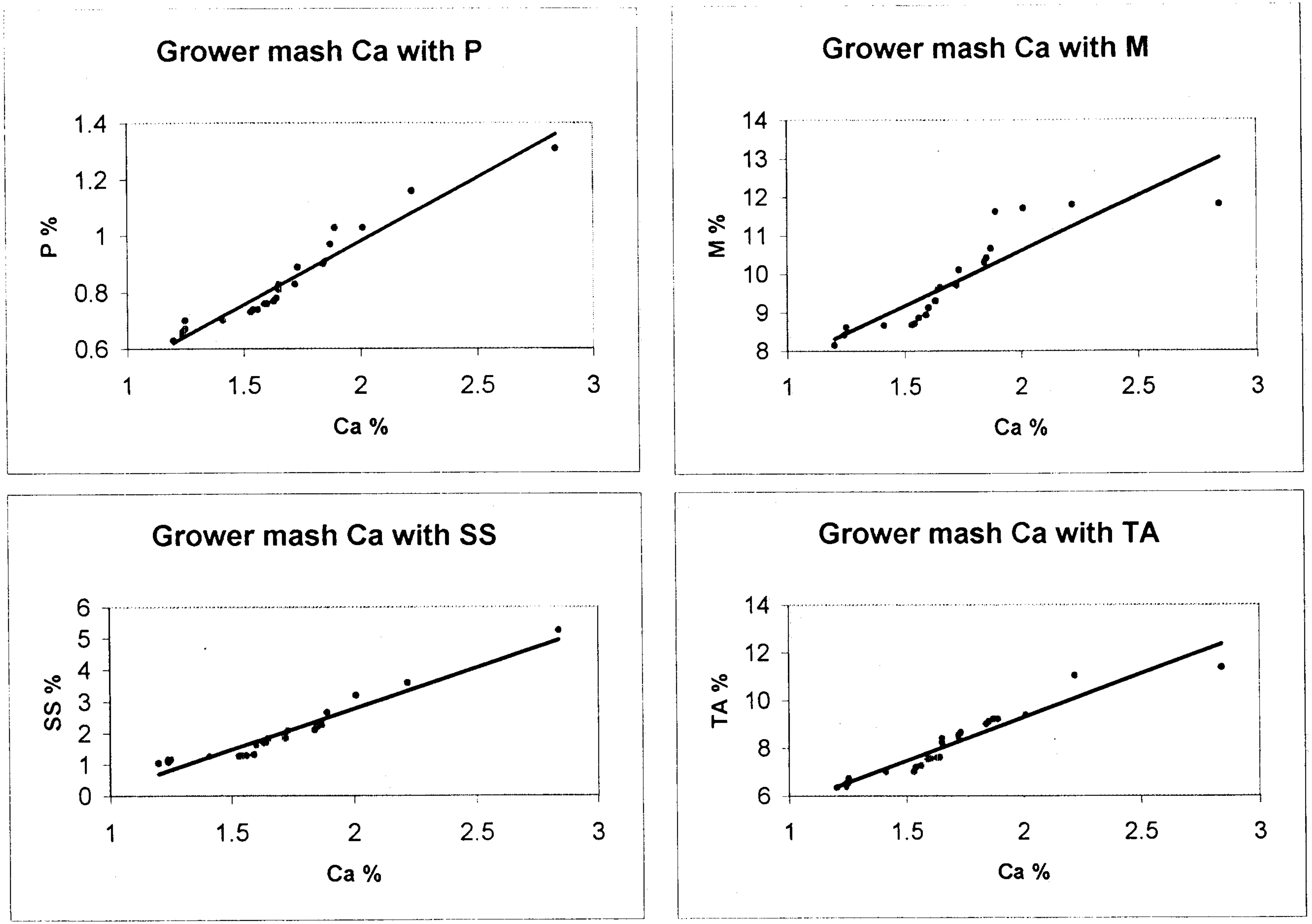


FIGURE - 7
CORRELATION OF Ca WITH P, M, SS AND TA IN CHICK MASH (CM)

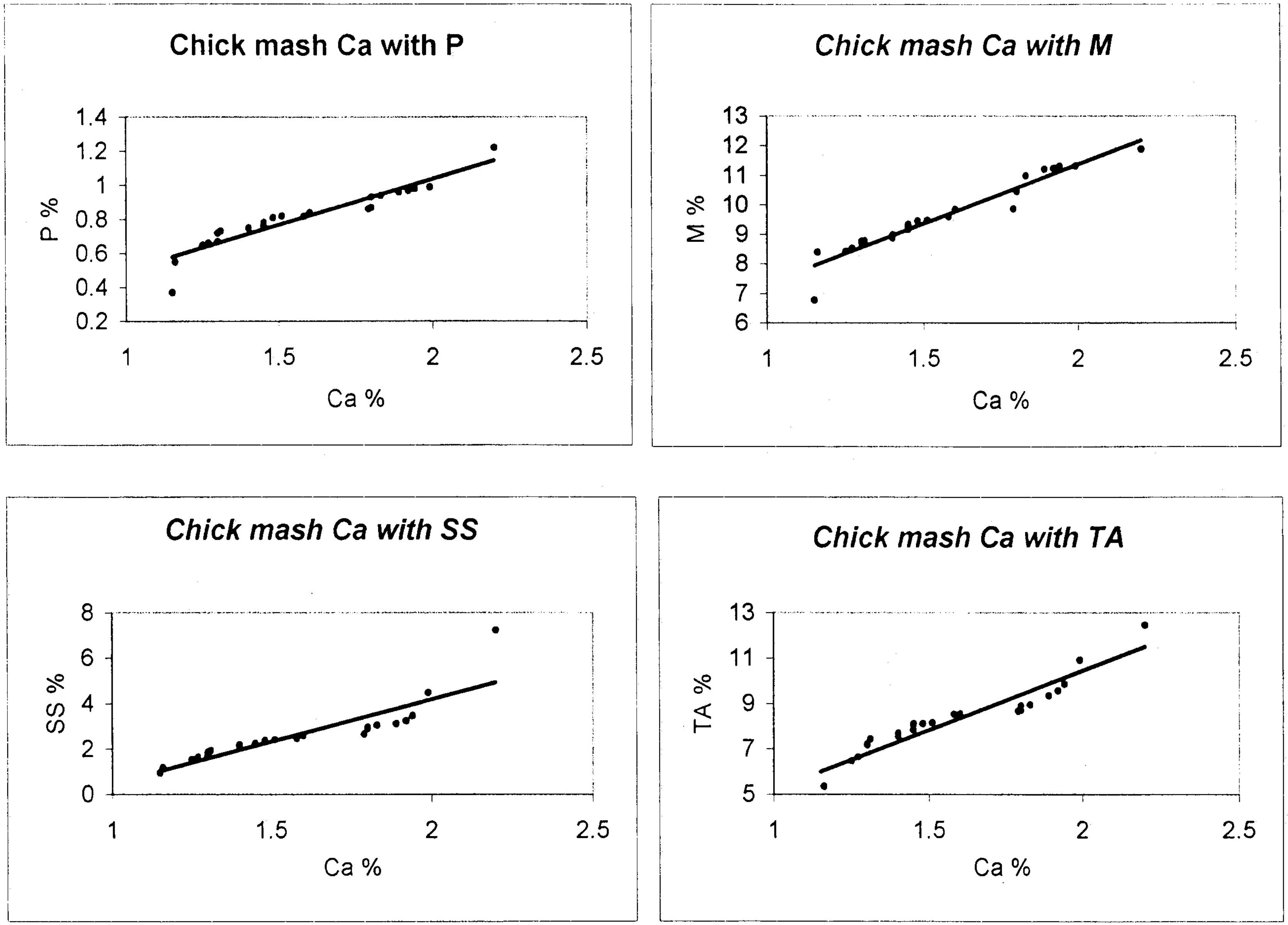


FIGURE - 8
CORRELATION OF Ca WITH P, M, SS AND TA IN BROILER STARTER MASH (BSM)

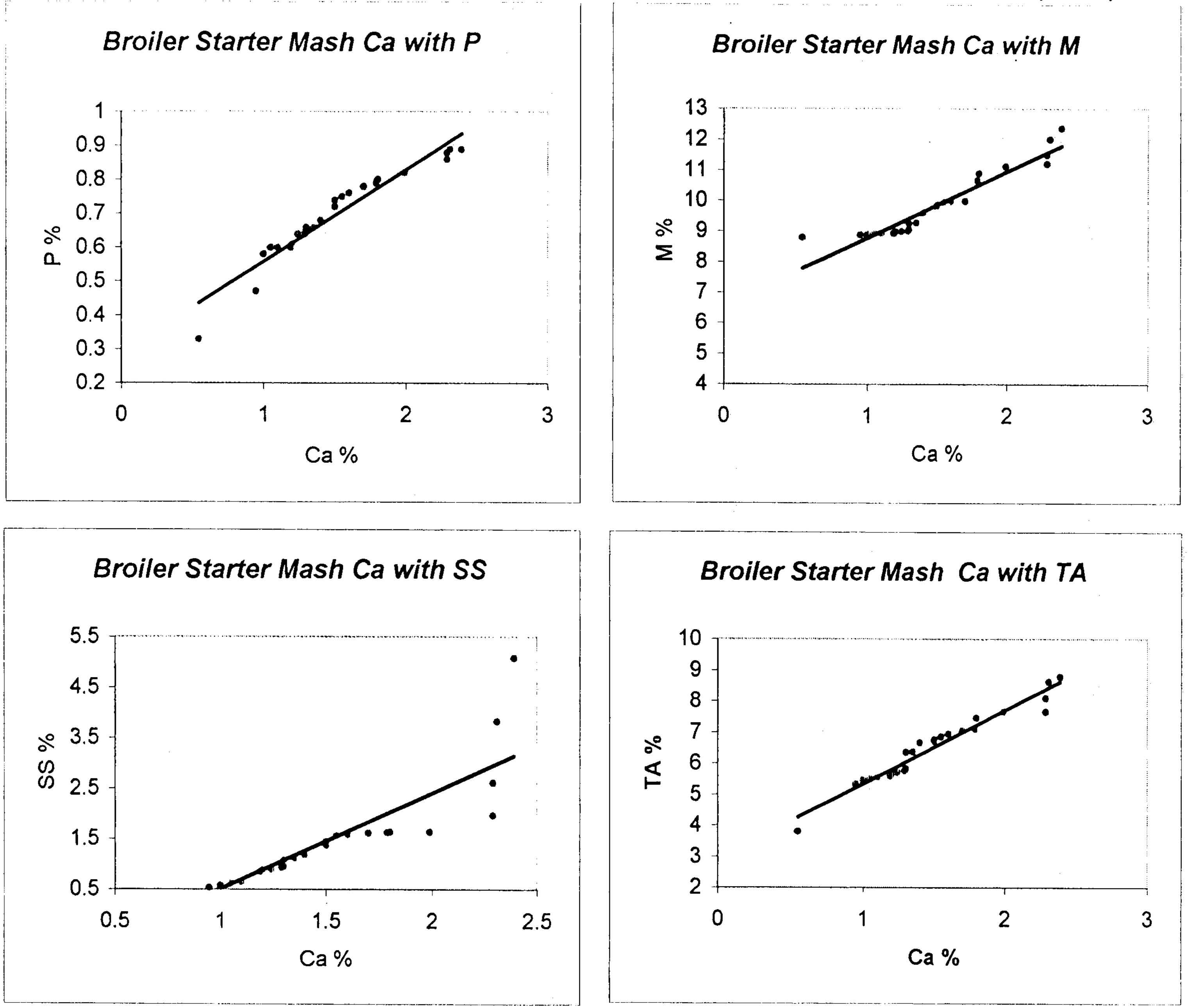


FIGURE - 9

CORRELATION OF Ca WITH P, M, SS AND TA IN BROILER FINISHER MASH (BFM)

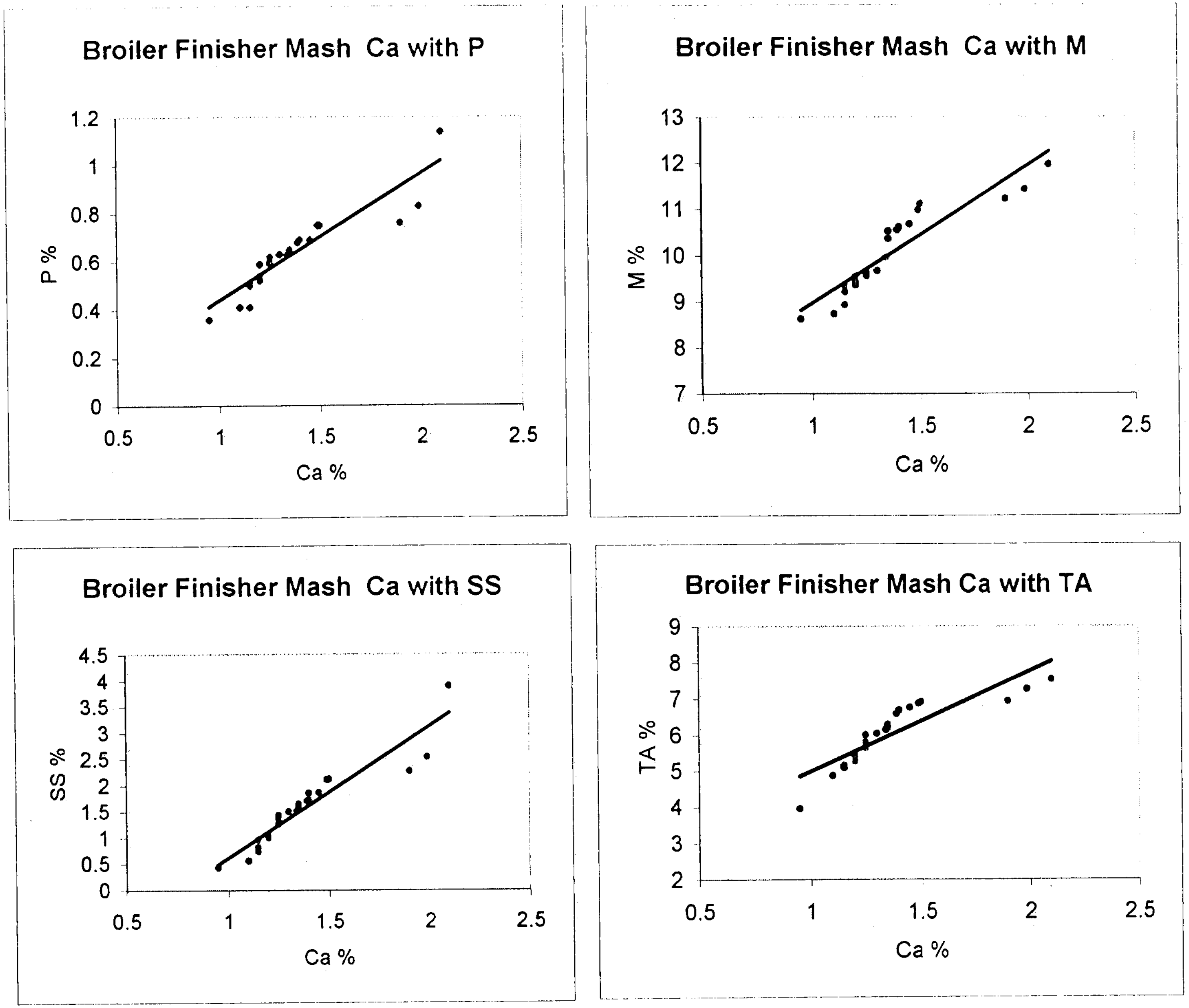


FIGURE - 10

CORRELATION OF P WITH M, SS, TA AND Ca IN LAYER MASH (LM)

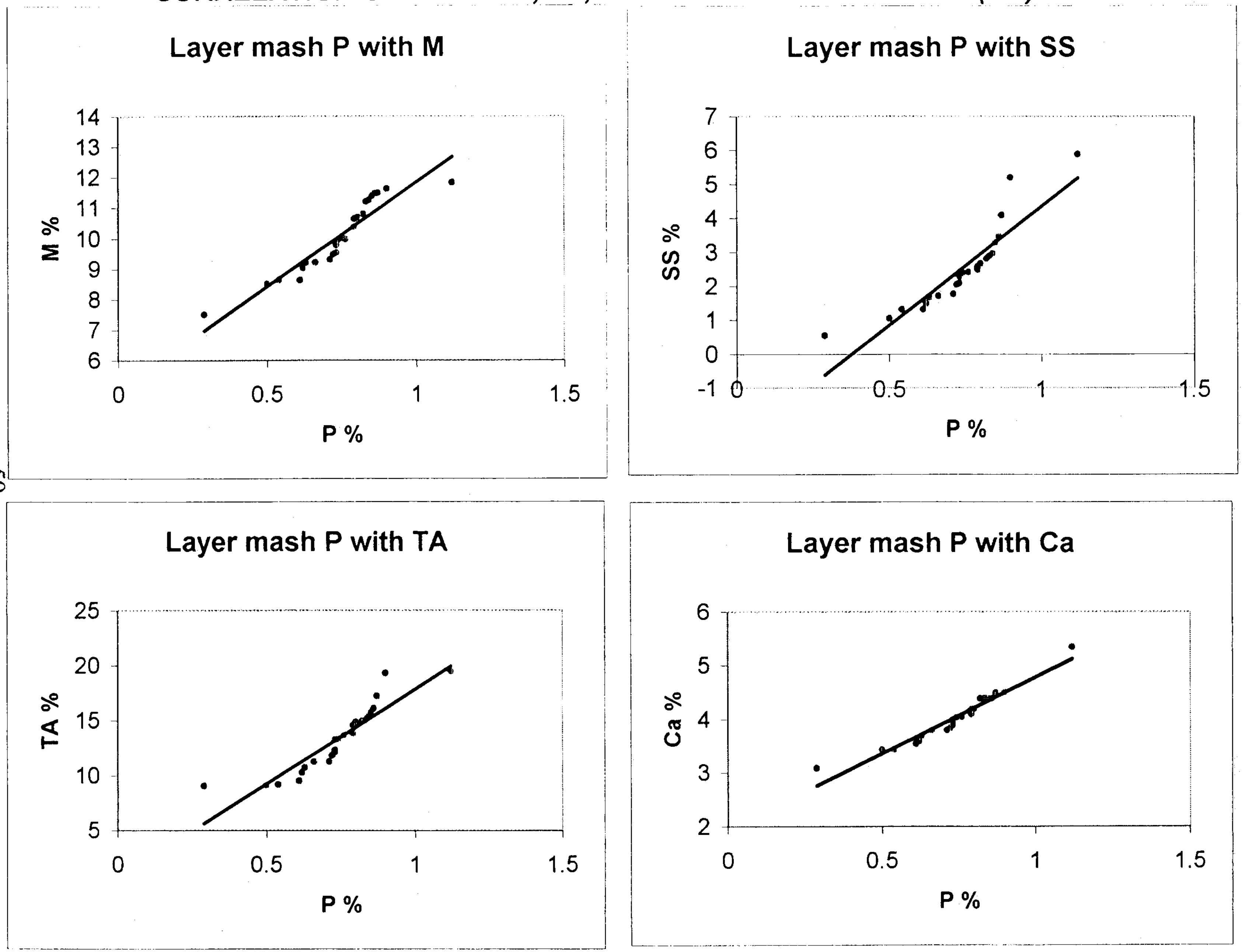


FIGURE - 11
CORRELATION OF P WITH M, SS, TA AND Ca IN GROWER MASH (GM)

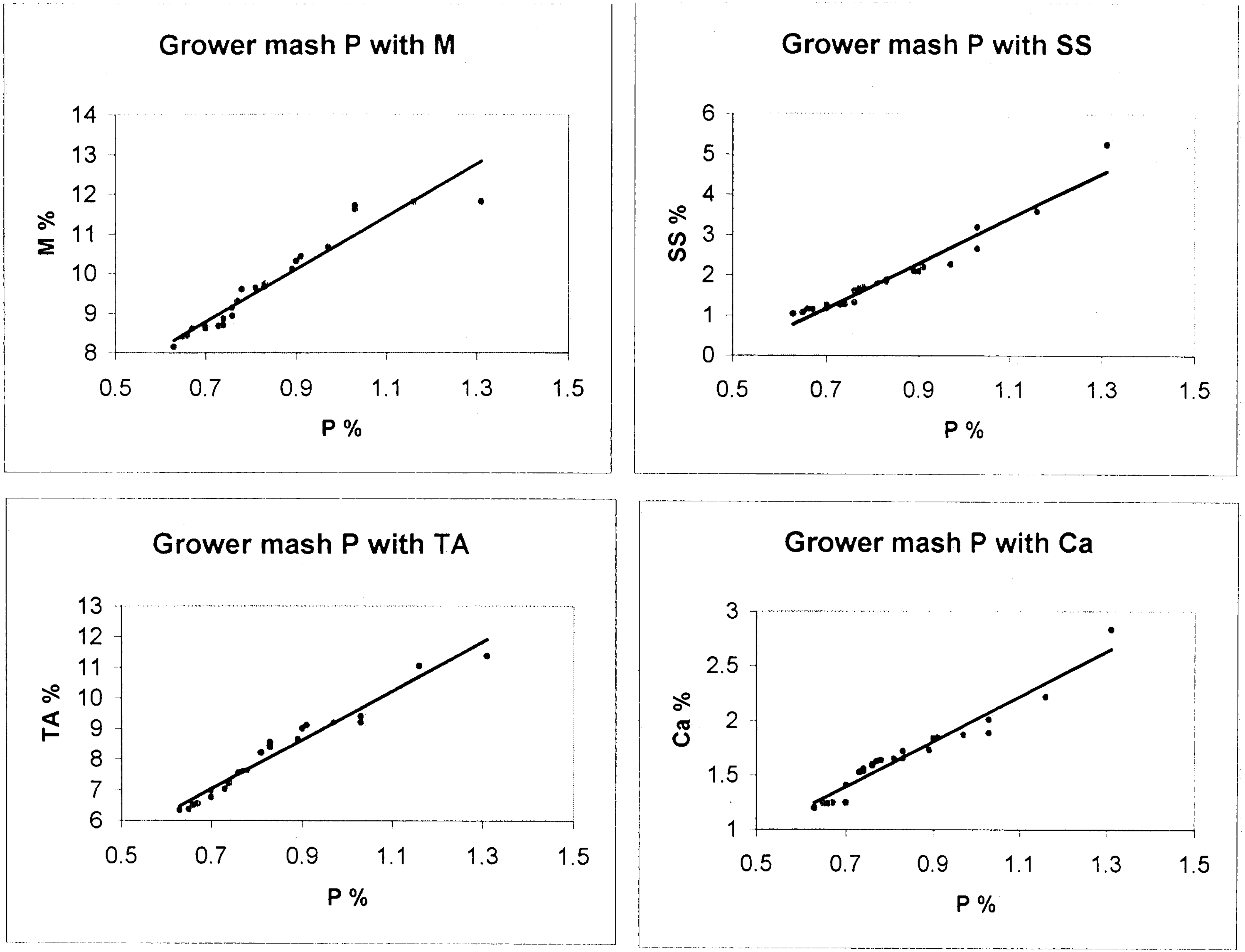


FIGURE - 12 CORRELATION OF P WITH M, SS, TA AND Ca IN CHICK MASH (CM)

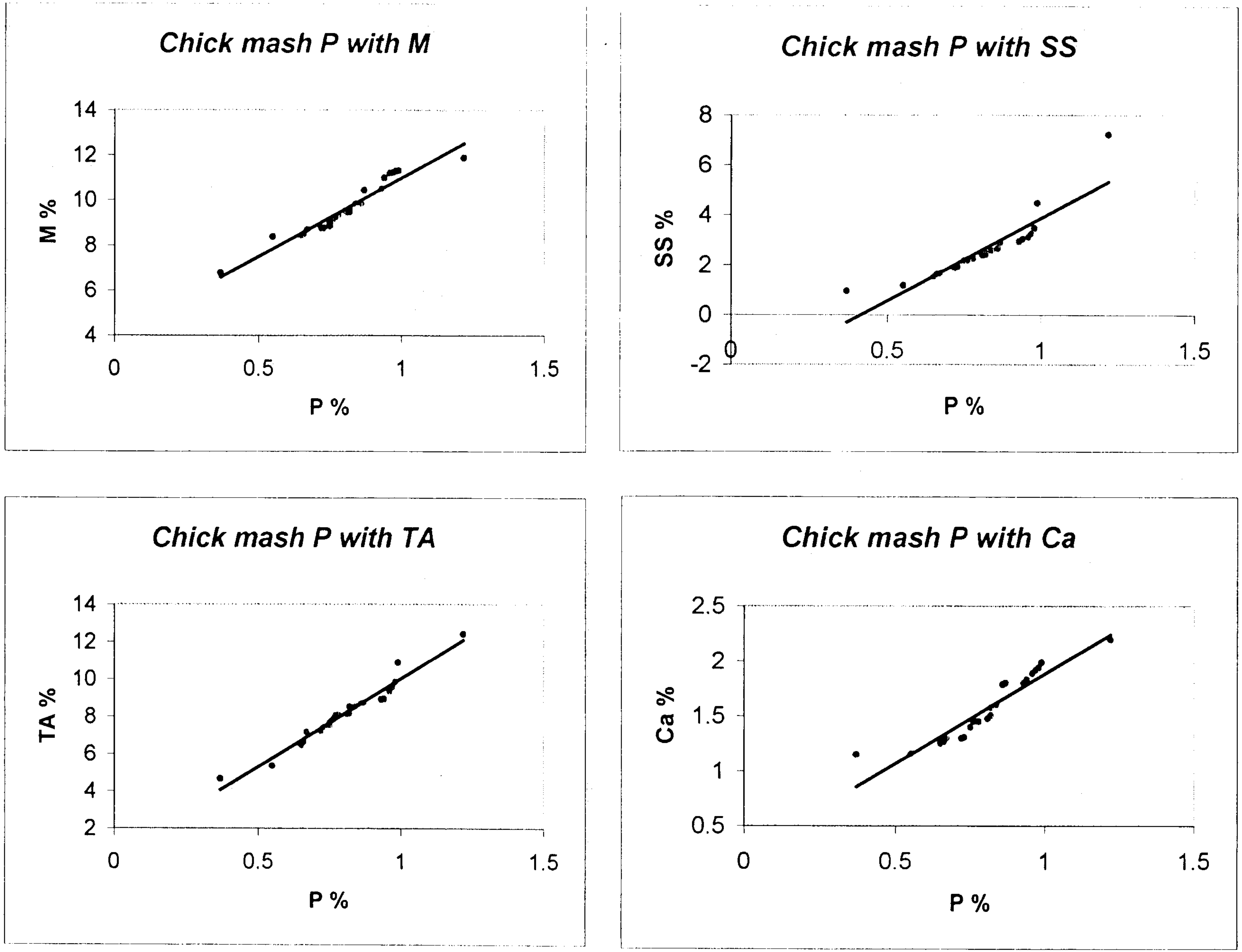


FIGURE - 13

CORRELATION OF P WITH M, SS, TA AND Ca IN BROILER STARTER MASH (BSM)

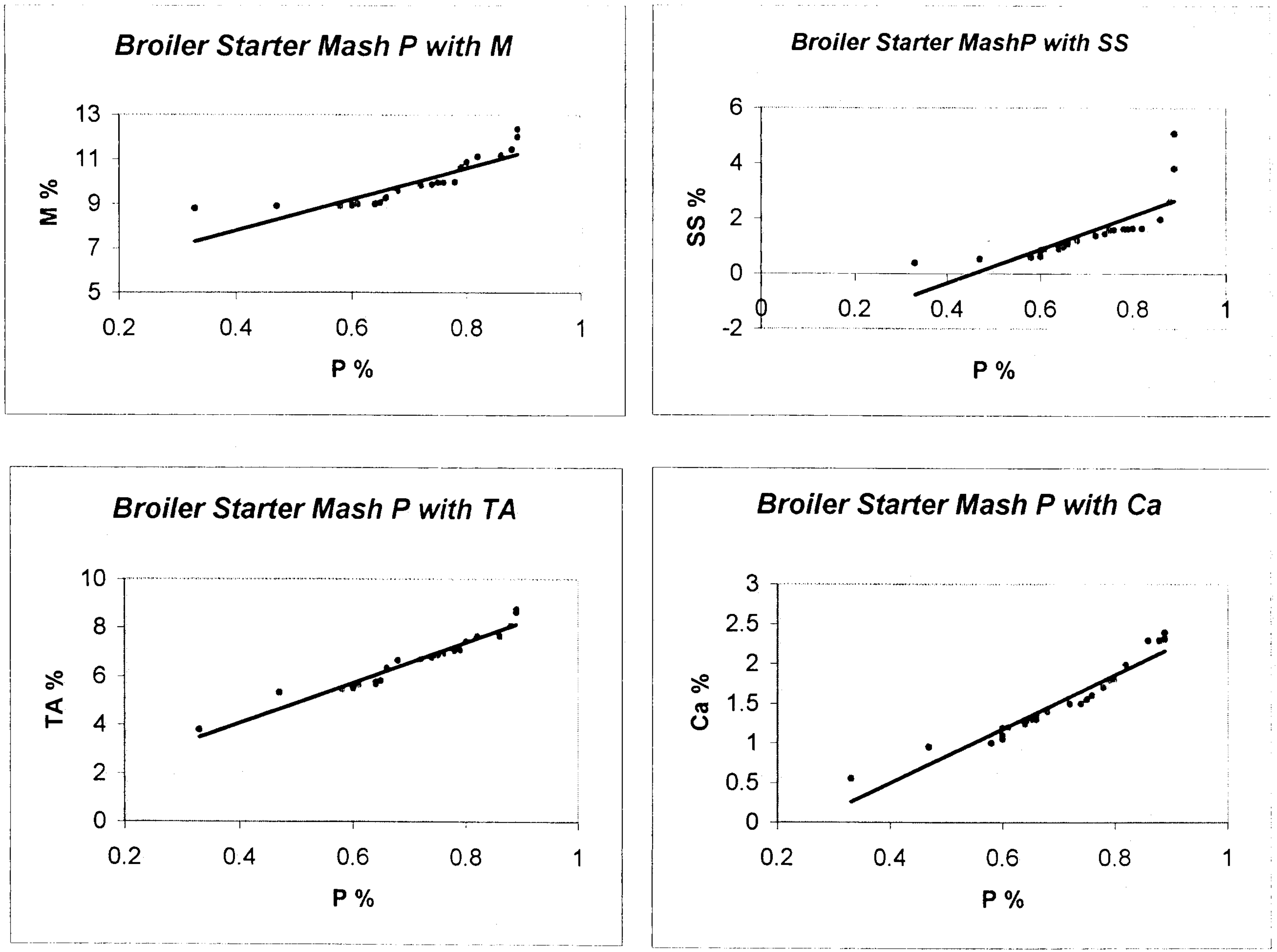


FIGURE - 14

CORRELATION OF P WITH M, SS, TA AND Ca IN BROILER FINISHER MASH (BFM)

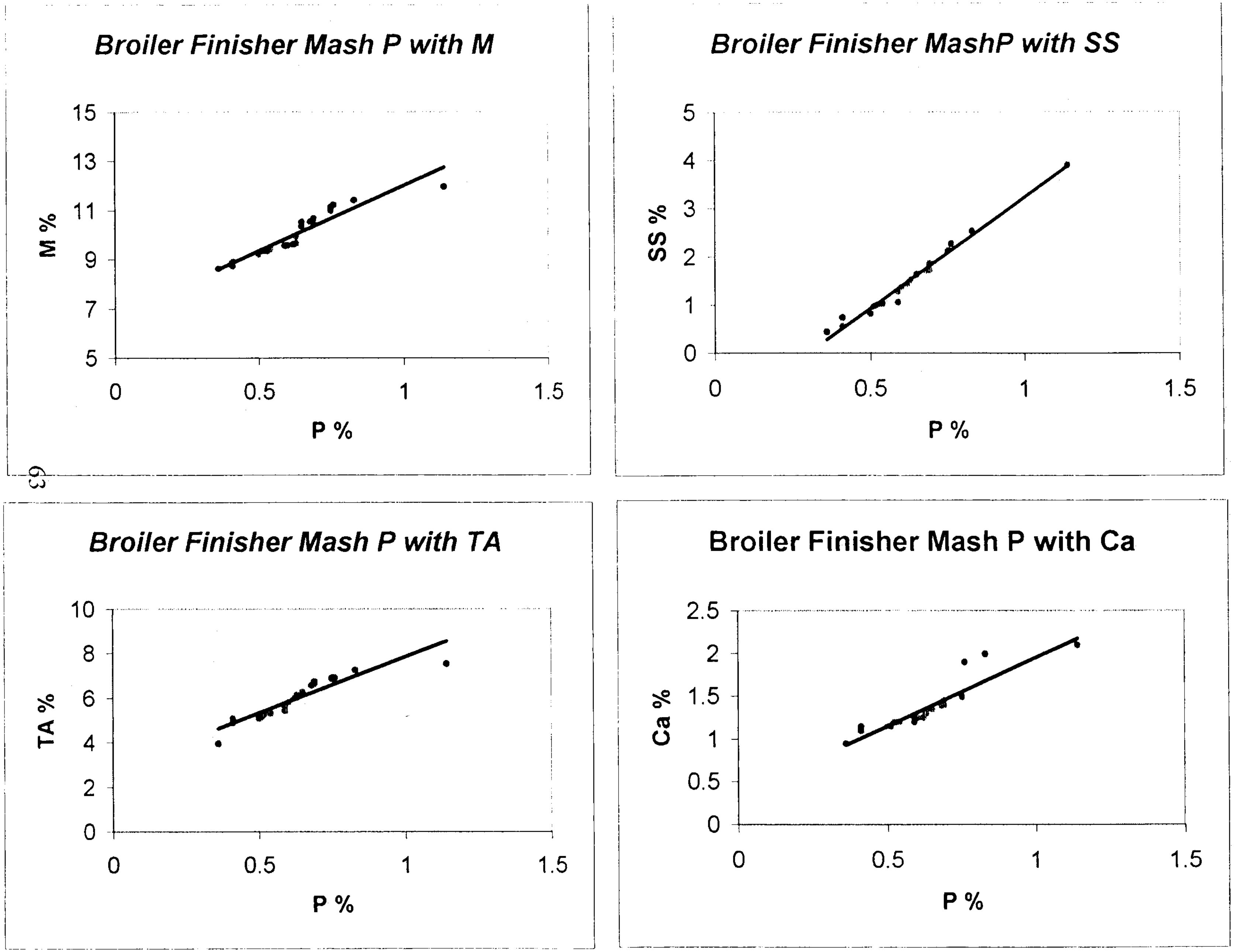


TABLE – 31

Correlation of Ca with P, M, SS and TA in Layer mash

S. No	*Calcium with other variables*	*Ratio of Ca with other variables*	*ISI ratio*
1	Ca : P	6.04:1 to 5.6:1	5:1
2	Ca : M	1:2.57 to 1:2.56	1:2.93
3	Ca : SS	1:1.14 to 1:1.125	1:1.06
4	Ca : TA	1:3.14 to 1:389	--

TABLE – 32

Correlation of Ca with P, M, SS and TA in Grower mash

S. No	*Calcium with other variables*	*Ratio of Ca with other variables*	*ISI ratio*
1	Ca : P	2:1 to 2.05:1	2:1
2	Ca : M	1:6.17 to 1:5.3	1:7.3
3	Ca : SS	1:1 to 1:1.38	1:2.67
4	Ca : TA	1:5 to 1:4.75	--

TABLE – 33

Correlation of Ca with P, M, SS and TA in Chick mash

S. No	*Ca with other variables*	*Ratio of Ca with other variables*	*ISI ratio*
1	Ca : P	1.88:1 to 2:1	2:1
2	Ca : M	1:6.3 to 1:5.63	1:7.3
3	Ca : SS	1:1.47 to 1:2	1:2.67
4	Ca : TA	1:5.17 to 1:5.13	--

TABLE – 34

Correlation of Ca with P, M, SS and TA in Broiler starter mash

S. No	*Ca with other variables*	*Ratio of Ca with other variables*	*ISI ratio*
1	Ca : P	1.7:1 to 2.1:1	1.6:1
2	Ca : M	1:9 to 1:6.67	1:9.17
3	Ca : SS	1:1 to 1.66:1	1:2.5
4	Ca : TA	1:45 to 1:4	--

TABLE – 35

Correlation of Ca with P, M, SS and TA in Broiler finisher mash

S. No	*Ca With other variables*	*Ratio of Ca with other variables*	*ISI ratio*
1	Ca : P	2.1:1 to 2.2:1	1.6:1
2	Ca : M	1;7.6 to 1:7	1:9.17
3	Ca : SS	1;1 to 1:1.7	1:2.5
4	Ca : TA	1:4.3 to 1:4.4	--

TABLE – 36

Correlation of Ca with P, M, SS and TA in Layer mash

S. No	*Phosphorus with other variables*	*Ratio of Phosphorus with other variables*	*ISI ratio*
1	P : M	1;14 to 1:12	1:14.67
2	P : SS	1:2 to 1:4.5	1:5.3
3	P : TA	1:18 to 1:17.5	

TABLE – 37

Correlation of Ca with P, M, SS and TA in Grower mash

S. No	*Phosphorus with other variables*	*Ratio of Phosphorus with other variables*	*ISI ratio*
1	P : M	1;12.5 to 1:11	1:14
2	P : SS	1:1.79 to 1:3	1:5
3	P : TA	1:10 to 1:9.5	--

TABLE – 38

Correlation of Ca with P, M, SS and TA in Chick mash

S. No	*Phosphorus with other variables*	*Ratio of Phosphorus with other variables*	*ISI ratio*
1	P : M	1:13 to 1:11	1:14.67
2	P : SS	1:2.8 to 1:4	1:5.3
3	P : TA	1:10.7 to 1:10	--

TABLE – 39

Correlation of Ca with P, M, SS and TA in Broiler starter mash

S. No	*Phosphorus with other variables*	*Ratio of Phosphorus with other variables*	*ISI ratio*
1	P : M	1:19 to 1:13.75	1:14.67
2	P : SS	1:1.67 to 1:3.13	1:4
3	P : TA	1:10 to 1:10	--

TABLE – 40

Correlation of Ca with P, M, SS and TA in Broiler finisher mash

S. No	*Phosphorus with other variables*	*Ratio of Phosphorus with other variables*	*ISI ratio*
1	P : M	1:19 to 1:14.66	1:14.67
2	P : SS	1:2 to 1:2.5	1:4
3	P : TA	1:11 to 1:9.3	--

TABLE – 41

Assessment of Ca: P ratio in various feed samples

S. No	*Name of the various feeds*	*Ca : P*	*ISI ratio*
1	Layer mash	6.04:1 to 5.6:1	5:1
2	Grower mash	2:1 to 2.05:1	2:1
3	Chick mash	1.88:1 to 2:1	2:1
4	Broiler starter mash	1.7:1 to 2.1:1	1.6:1
5	Broiler finisher mash	2.1:1 to 2.2:1	1.6:1

The correlation ratio of Ca, P, TA, SS and M for different feed samples along with ISI standard ratio are shown in the Table (31-41) and are explain as follows.

Assessing the ratio of Ca : P and Ca : other variables in various feeds such as LM, GM, CM, BSM and BFM from the experimental values and values obtained from the graph could reflect the following points .

- The ratio of C : P in various feeds was greater than ISI requirements.
- The high content of Ca was found in different feeds manufactured from the different processing units
- The ratio of Ca with other variables such as moisture and sand and silica could bring the information that the quantity of moisture and sand and silica was less than the ISI requirement.

The results were found to be on par with the earlier reports, C.G. Belyavin and Marnago could bring out the relationship between P and shell formation. According to them, P is an important element for shell formation not because of shell contains much P but because of the special relationship between Ca and P in bone formation. Ca is stored in the phosphatase skeleton probably almost entirely as calcium phosphate. If Ca is used for shell formation then P must be excreted.

Though the literature insisted on maintaining Ca : P ratio as given by ISI requirement there were supporting the importance high dietary Ca which was essential for the increase in egg specific gravity. Ousterhout 1980, and Roland 1990, Abdualla *et al.*, 1993, reflected the same idea.

The low values of moisture and SS may be attributed to the least important nature of SS and M in feeds. High levels of SS in the diet of farm animals may be detrimental.

TA content was found to be high in various feeds. Many workers observed linear relationship between dietary P and TA content by Mikalein and Sell (1982) reported that increased P level in the diet of layers increased tibial ash content from

57.30 to 59.81 %. The linear relationship between dietary P level and TA was also observed by Said *et al* (1984) and Hurwitz (1984), Frost *et al* (1990) and Rao *et al* (1992). An ash value higher than normal is generally associated with contamination by sand.

FIGURE - 15
STANDARD VALUES OF Ca, P, SS AND M Vs. EXPERIMENTAL VALUES OF Ca, P, SS AND M IN LAYER MASH

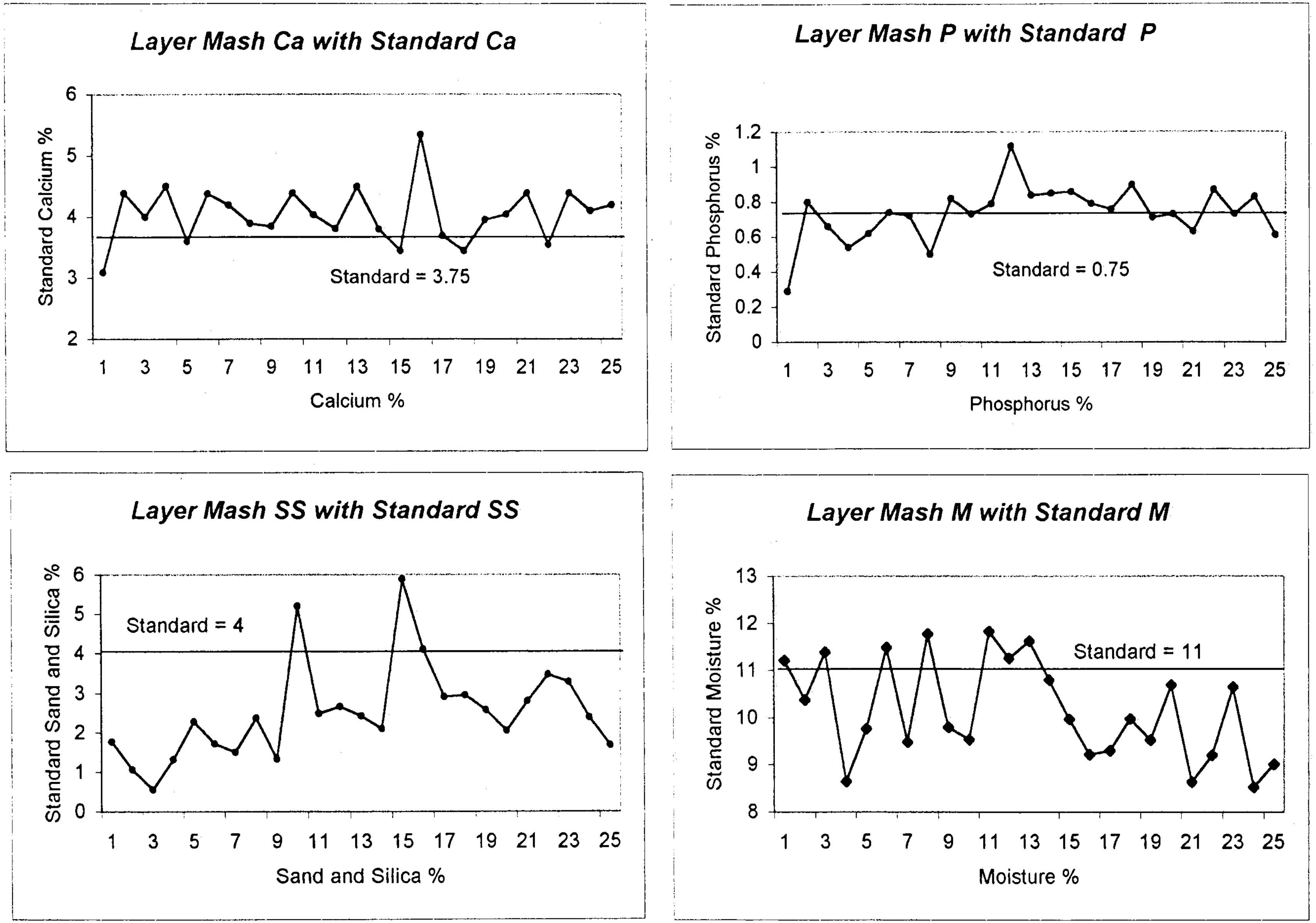


FIGURE - 16
STANDARD VALUES OF Ca, P, SS AND M Vs. EXPERIMENTAL VALUES OF Ca, P, SS AND M IN GROWER MASH

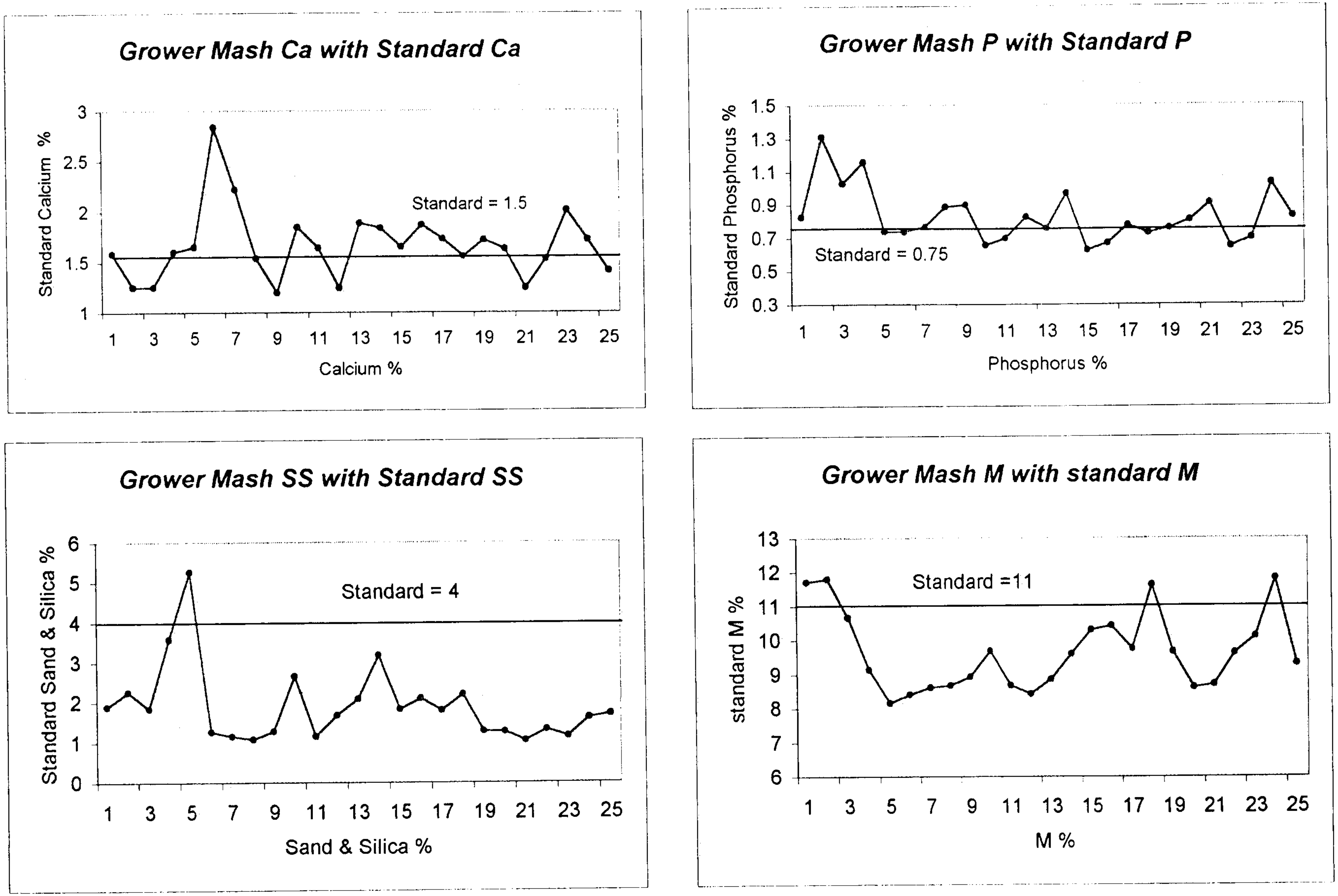


FIGURE - 17
STANDARD VALUES OF Ca, P, SS AND M Vs. EXPERIMENTAL VALUES OF Ca, P. SS AND M IN CHICK MASH

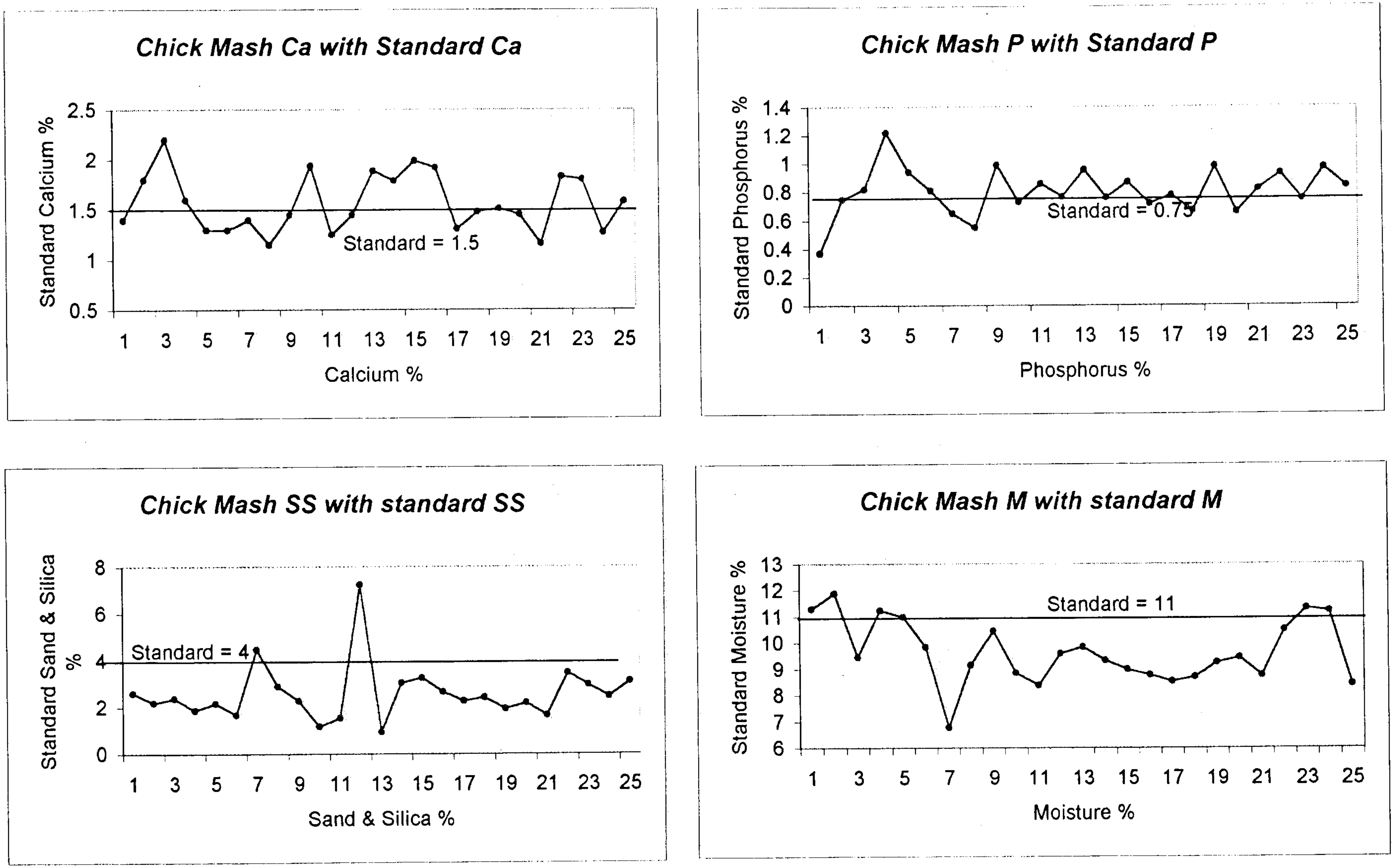


FIGURE - 18
STANDARD VALUES OF Ca, P, SS AND M Vs. EXPERIMENTAL VALUES OF Ca, P, SS AND M IN BROILER STARTER MASH

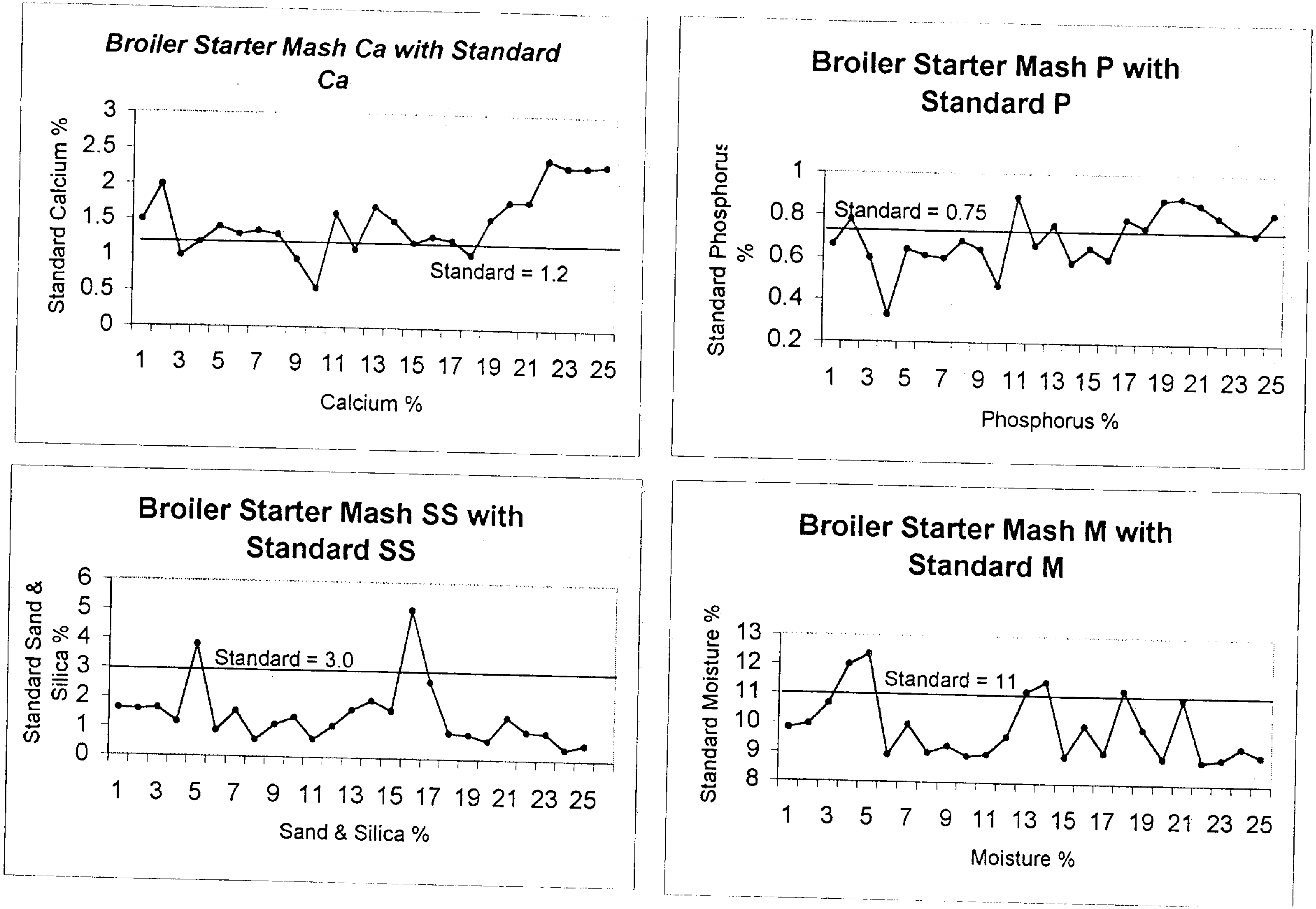
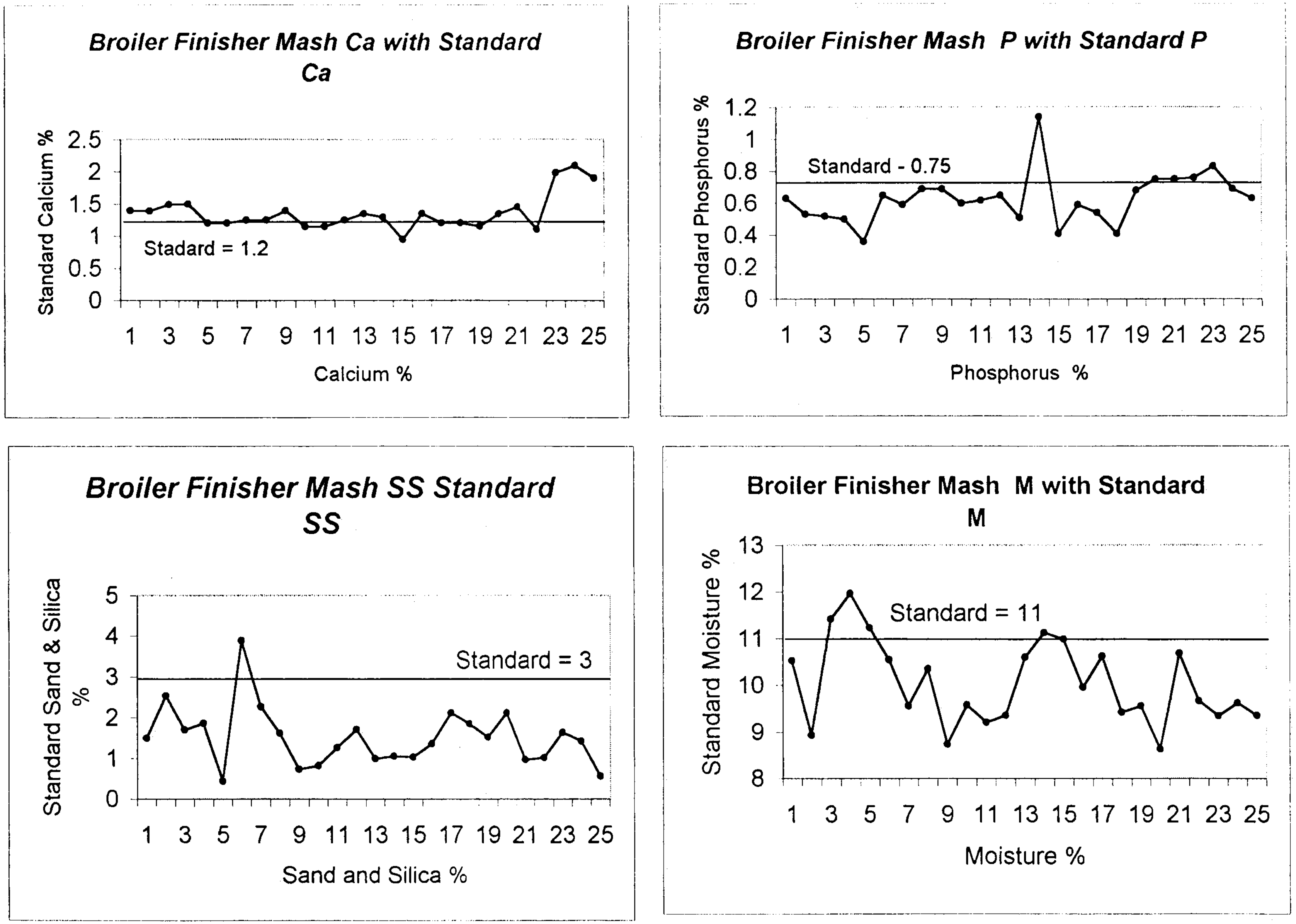


FIGURE - 19

STANDARD VALUES OF Ca, P, SS AND M Vs. EXPERIMENTAL VALUES OF Ca, P, SS AND M IN BROILER FINISHER MASH



Standard values of Ca, P, SS, M Vs Experimental values of Ca, P, SS, M present in various feed samples

The experimental values of Ca, P, SS and M plotted against the ISI values of Ca, P, SS and M for the various feed samples which are shown in the figure (14- 18).

Comparison of the amount of Ca, P, M, SS and TA present in various feeds such as LM, GM, CM, BSM and BFM with the respective standard values were presented in the Table-30 and very well depicted in the graphs.

Graphical representation and values presented in the table (31-41) could infer that in all varieties the Ca and P content was greater than the ISI requirement. This information is on par with the findings of C.G. Belyavin and Marango (1987). According to him P is an important element for shell formation not because of eggshell contains much P but because of the special relationship between Ca and P in the bone formation. Ca is stored in the skeleton probably almost entirely as Ca and P. If Ca is used for shell formation, then P must be excreted. This result also support the finding of Roland (1985) who suggested that no minerals are more important to commercial egg producers in maintaining eggshell quality than Ca and P. It was also reported that a high level of either Ca or P reduces the efficiency of utilization of other and can also reduce the utilization of other minerals.

J.B Hess and W.M. Britton also reported that hens fed low P had lower egg production and lower feed consumption than the hens fed higher levels of P. Body weight was lower in hens fed the two lower levels of P. Percentage egg shell was lower than the contracts when fed low P.

SUMMARY AND CONCLUSION

CHAPTER V

SUMMARY AND CONCLUSION

With a view to assessment and correlation study of macro (Ca and P) and micro nutrients (SS, TA, M) in feeds manufactured in various processing units at Namakkal district. Twenty five samples each from LM, GM, CM, BSM and BFM were collected and the samples were analysed for Ca, P, TA, SS and M. The data were subjected to statistical tools like mean, correlations and multiple regressions.

The findings are furnished below

Calcium and phosphorus content was more in LM, GM, CM, BSM and BFM.

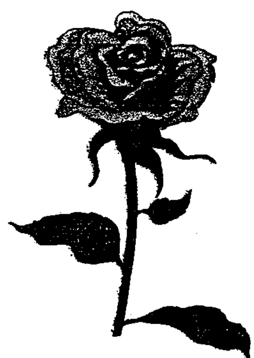
- C : P was found to be slightly higher than the ISI requirements in LM, GM, CM, BSM and BFM.
- Data showed the high values of TA in different feeds were recorded
- Experimental evident could infer that moisture content and sand and silica were less than the standard values in various feeds.
- Correlation studies of P and TA revealed no significant association. M claimed a positive significant association of LM with BFM. Calcium content of BSM showed a positive significant with BFM. Sand and silica content of GM indicated the positive significant with BSM.
- Regression estimates exhibited a non significant influence. A positive significant influence was exerted by Ca with TA in Layer Mash. An impressive positive significant influence was encountered by Ca with P, others had a negative significance.
- The normal practice of phosphorus feeding needs modification in order to provide the optimum levels of non phytin phosphorus, so that the cost of feeding can be reduced appreciably, in addition to reduce the ill effects of

excess dietary P and also environmental pollution. The optimum levels of non phytin phosphorus for commercial broilers and layers is about 0.45 and 0.2 % respectively. Use of P at an level to meet the birds requirement not only reduces the cost of production, also eliminates the ill effets of excess dietary P on birds performance and ensures environmental safety.

- Conclusion to be drawn that egg shells treated with phosphoric acid can be used as a calcium source in laying hens. It can replace commonly used sources, providing phosphorus content is taking into account when formulating the diet.

- Even though calcium is one of the cheapest feed ingredients in a layer feed, it can one of the most costly ingredient if improperly used. Feeding inadequate calcium can increase feed cost, feed intake, body weight, liver weight, liver fat, shell problems and skeletal problems. All of which can have significant adverse effect on producer profits.

- Due to cost consideration and local availability, the mineral mixture manufactures select calcium and phosphorus supplements, not in the form of pure chemical compounds but in the form of locally available ores or others for blending the name into mineral mixture.



BIBLIOGRAPHY

BIBLIOGRAPHY

Agarwal,B.L., and Talapatra, S.K., (Jan 1971). Biological availability of calcium in feedstuffs. Availability of calcium from mineral salts. Jr. Indian Journal of Animal Science, 41, 11-14.

Baker, D.H., Biehl, R.R. and Emmert, J.L., (1998). Vitamin D_3 requirement of young chicks receiving diets varying in Calcium and available phosphorus. Jr. British Poultry Science, 39, 413-417.

Belyavin, C.G., and Marangos, A.G., (1987). Natural products for egg yolk pigmentation. Animal nutrition, 238-275.

Bingfan Zhang and Craig.N.Coon (1997). The relationship of Calcium intake, source, size, solubility in vitro and in vivo and gizzard limestone retention in laying hens. Jr. Poultry Science, 27, 1702-1706.

Bondi., (1989). The nutritional importance of minerals, Animal nutrition, 212.

Carlos, A.B., and Edwards, H.M., (1998). The effects of 1,2 dihydroxy cholecalciferol and phytate Phosphorus utilization by laying hens. Jr. Poultry Science, 77, 850-858.

Cartes, S.D., and Cromwell, G.C., (1998). Influence of porcine somatotropin on the phosphorus requirement of finishing pigs, performance and bone characteristics. Jr. Animal Science, 76, 584-595.

Christensen, V.L., and Edens, F.W. (1985). Magnesium, calcium and phosphorus content of shells from hatching and non hatching Turkey eggs. Jr. Poultry Science, 64, 1020-1027.

Clunies, M., Parks, D. and Leeson, S., (1992). Calcium and phosphorus metabolism and eggshell thickness in laying hens producing thick or thin shells. Jr. Poultry Science, 71, 490-498.

Clunies, M., Parks, D., and Leeson, S., (1992). Calcium and phosphorus metabolism and eggshell formation of hens fed different amounts of calcium, Poultry Science, 71, 482-489.

Crowley, T.A., Kurnick, A.A., and Reid, B.L., (1963). Dietary phosphorus for laying hens, Jr. Poultry Science, 42, 758-765.

Dr. David A. Roland., (1998). Calcium feeding of commercial layers Jr. Poultry Fortune, 18.

Davidson, J., and Boyne, A.W., (1970). The Calcium and phosphorus requirements of laying hens. Jr.British Poultry Science, 11, 231-240.

Edwards, H.M., (1983). Phosphorus I Effect of breed and strain on utilization of sub-optimal levels of phosphorus in the ration. Jr. Poultry Science, 62, 77-84.

Garlich, J.D., Edens, F.W., and Parkhurst, C.R., (1978). The phosphorus requirement of laying hens with special reference to high environmental temperature.
Jr.world Poultry, 598-609.

Gillis, M.B., Norris, L.C., and Henser, G.F., (1949). The effect of phytin on the phosphorus requirement of the click. Jr. Poultry Science, 28, 283-292.

Gordon, R.W., and Roland, D.A., (1998). Influence of supplemental phytase on Calcium and Phosphorus utilization in laying hens. Jr. Poultry Science, 77, 290-294.

Guinotte, F., and Nys, Y., (1991, March-19). Effects of particle size and origin of Calcium sources on eggshell quality and bone mineralization in egg laying hens. Jr. Poultry Science, 70, 583-592.

Guinotte, F., and Nys, Y., (1991). The effects of particle size and origin of Calcium carbonate on performance and ossification characteristics in Broiler chicks. Jr. Poultry Science, 70, 1908-1920.

Haresign ,W., Cole, D.J.A., (1988). Minerals and trace elements requirement of poultry. Recent advances in Animal nutrition, 100-102.

Hess, J.B., and Britton, W.M., (1997). Effects of Dietary magnesium excess in white leghorn hens. Jr. Poultry Science, 76, 703-710.

Hopkins, J.R., Ballantyne, A.J., and Jones, J.L.O., (1987). Dietary Phosphorus for laying hens. Animal Nutrition, 231-237.

Jain, R.K., and Chopra, R.C., (1998, May 15). Influence of Dietary Phosphorus inadequacy on concentration of inorganic phosphorus, calcium, magnesium in plasma, saliva and rumen liquor in calves. Jr. Indian Journal of Animal nutrition, 2, 83-87.

Jerry.L., (1994). Minerals and vitamins. Nutrient requirement of poultry, 33.

Jonathan. M. Naylor., and Sarah. L. Ralston., (1989). Calcium, phosphorus and magnesium – Basic principle Book – Large Animal clinical nutrition, 40-52.

Keshavarz, K., (1991, December-3). The effect of Calcium sulphate in combination with different sources and forms of Calcium carbonate on acid – base balance and eggshell quality. Jr. Poultry Science, 70, 1723-1731.

Keshavarz, K., (1998). Further investigations on the effect of Dietary manipulation of protein, Daily requirement for laying hens. Jr. Poultry Science, 77, 1333-1346.

Keshavarz, K., (1998). Reducing protein, calcium and phosphorus of laying hens. Commercial poultry nutrition, 1334-1346.

Keshavarz, K., (1998). Investigation on the possibility of reducing protein, phosphorus and calcium requirements of laying hens by manipulation of time of access of these nutrients. Jr. Poultry Science, 77, 1320-1332.

Lall, D., Kapoor, V., and Prasad, T., (1998). Sources, compositional merit and criteria for selection of different calcium and phosphorus supplements for Livestock feeding. Jr. International Journal of Animal Science, 13, 25-32.

Mendonca, C.X., Alvarez, J.C., Garzillo, J.M.F., Ghion, S.E., and Leal, P.M., (1997). Biological Evolutions of commercial dicalcium phosphates as sources of available phosphorus for Broiler Chicks. Jr. Poultry Science, 76, 1707-1713.

Patrick, S ., (1980). Feeding programs. Poultry feeds and nutrition, 417-421.

Philip, J., (1976). Nutrient interrelationship. Poultry feeds and nutrition, 250-254.

Rama Rao, S.V., and Nagalakshmi, D., (March 1997). Are we feeding Excess phosphorus to poultry Jr. Poultry Fortune , 10-11.

Rawashdeh, O.F., Lafi, S.Q., Abdul-Aziz, T.A., and Ereiffi, K.I., (1995, October). Effects of starvation on serum levels of calcium phosphorus, uric acid and creatinine in newly hatched broiler chicks. Jr. Indian Journal of Animal Sciences, 65, 1081-1085.

Russel, Lee., and Dowell, M.C., (1992). Calcium and phosphorus minerals in Animal and human Nutrition, 26-77.

Said, N.W., Sunde, M.L., Bird, H.R., and Suttie, J.W., (Aug-28, 1979). Raw rock phosphate as a phosphorus supplement for growing pullets and layers. Jr. Poultry Science, 58, 1557-1563.

Salman, A.J., Ali, M.S., and Ginnis, J. Mc., (1969). Effect of level and source of phosphorus and different calcium levels on productivity and phosphorus utilization by laying hens. Jr. Poultry Science, 48, 1004-1009.

Shafey, T.M., and Mc. Donald, H.W., (1991). The effects of Dietary calcium phosphorus and protein on the performance and nutrient utilization of Broiler Chickens. Jr. Poultry Science, 70, 548-553.

Shivaram, K. Rao., David, A. Roland and Joseph, I. Orban., (1991, Jan, 7). Influence of Dietary cholecalciferol, calcium and phosphorus on urinary calcium in commercial leghorn hens. Jr. Poultry Science, 70, 1921-1927.

Simons, P.C.M., and Versteegh, H.A.J., (June, 1990). Phytase in feed reduces phosphorus excretion. Jr. Poultry, 15-17.

Singh, R.P., and Talapatra, S.K., (January, 1977). Utilization of Calcium as influenced by varying levels of dietary protein in young Hariana calves. Jr. Indian Journal of Animal Science, 41, 9-11.

Stanislaw, M., Waldemar, K., and Grazyna, B., (1990). The retention of phytin phosphorus from diets with fat supplements in broiler chicks. Jr. Animal feed Science and Technology, 31, 223-230.

Stevon Leason., and John, D. Summers., (1990). Nutrition and shell quality commercial Poultry Nutrition, 117-118.

Swaminathan, M., (1989). Principles of nutrition and Dietetics. 1, 190-193.

APPENDICES

APPENDIX – I

ESTIMATION OF CALCIUM

Principle

The calcium in the sample was precipitated as calcium oxalate using ammonium oxalate in acid medium. The precipitated calcium oxalate was filtered out, washed with ammonium hydroxide to free ammonium oxalate from the precipitate and dissolved in hot sulphuric acid and the liberated oxalic acid was estimated by permanganimetric titration.

$$CaC_2O_4 + H_2SO_4 \longrightarrow CaSO_4 + H_2C_2O_4$$

$$H_2C_2O_4 + (O\ ) \longrightarrow H_2O + 2CO_2$$

Apparatus

Silica crucible, glass rod, burette, pipette, whatmann no-42 filter paper, volumetric flask 100 ml, conical flask.

Reagents

i) ***Ammonium oxalate***

Dissolved 4.2 g of ammonium oxalate to get 100 ml of the solution.

ii) ***Ammonium hydroxide (1:3)***

Dissolved 100 ml of liquor ammonia in 300 ml of dissolved water.

iii) ***Hydrochloric acid (1:3)***

Dissolved 100 ml of HCl in 300 ml of distilled water.

iv) ***Nitric acid (1:3)***

Dissolved 100 ml of nitric acid in 300 ml of distilled water.

(v) ***Sulphuric acid (1:4)***

Dissolved 100 ml of sulphuric acid in 400 ml of distilled water.

(vi) ***Ammonium hydroxide (1:50)***

Dissolved 1 ml of liquor ammonia in 50 ml of distilled water.

(vii) ***Potassium permanganate (0.1N)***

Dissolved 3.16 ml of $KMnO_4$ in minimum quantity of water. Boiled for 10 minutes to oxidise any organic carbon present, cooled and made upto the mark in a volumetric flask. Standardise using 0.1N $H_2C_2O_4$ and heated upto 60°C.

APPENDIX II

ESTIMATION OF PHOSPHORUS

Principle

Phosphorus in the sample is converted to phosphomolybdic acid by adding ammonium molybdate. Phosphomolybdic acid in presence of a reductant sodium metabisulphite, sodium sulphite and 1- amino-2-naphthol-4-sulphonic acid (ANSA) gives a blue colour whose intensity was measured photometrically using a wavelength of 660nm.

Reagents

(i) Hydrochloric acid

Dissolved 10 ml of concentrated hydrochloric acid in 30 ml of distilled water.

(ii) Nitric acid

Dissolved 10 ml of concentrated nitric acid in 30 ml of distilled water.

(iii) 1-amino-2-naphthol –4-sulphonic acid

Dissolved 0.025 g of ANSA in 9.75 ml of 15% Sodium metabisulphite and 0.25 ml of 20% sodium sulphite in a test tube, mixed thoroughly and filtered through whatmann No1.

(iv) Ammonium molybdate solution

Dissolved 2.5 g of ammonium molybdate in 20 ml of distilled water and added 8.23 ml of concentrated sulphuric acid and made upto 100 ml with distilled water (store in a dark bottle).

(v) Phosphorus standard

Dissolved 0.0353 g of KH_2PO_4(Potassium dihydrogen orthophosphate) in distilled water and made upto 100 ml which gives 80 µg of phosphorus (stock solution). Added 1 ml of chloroform to prevent mold growth for long storage.

(i) 0.1 ml of the stock solution was taken and added 2.9 ml distilled water 1 ml of ammonium molybdate solution and 0.4 ml of ANSA Solution.

(ii) Take 0.2 ml of the stock solution added 2.8 ml of distilled water, 1 ml of ammonium molybdate solution and 0.4 ml of ANSA solution and measure optical density at 660 nm which corresponds to 16 μg of standard phosphorus.

APPENDIX III

ESTIMATION OF ACID INSOLUBLE ASH

(SAND AND SILICA)

Principle

The total ash was dissolved in dilute acids when all mineral except sand and silica go into solution. The solution was filtered off and the residue left behind was ignited, cooled and weighed to get the acid insoluble ash.

Apparatus

Balance, weight box, whatmann filter paper no-42, funnel, watch glass, volumetric flask, desiccator and muffle furnace.

Reagents

(i) Hydrochloric acid (1:3)

Dissolved 100 ml Hcl in 300 ml of distilled water.

(ii) Nitric acid (1:3)

Dissolved 100 ml of nitric acid in 300ml of distilled water.