THE EFFECTS OF PROTEIN SOURCE AND LEVEL OF INORGANIC PHOSPHOROUS ON LAYING HEN PERFORMANCE AND PHOSPHOROUS BALANCE

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Michael Bernd Schalm

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ABSTRACT

Schalm, Michael Bernd. M. Sc., The University of Manitoba, October, 1989. The Effects of Protein Source and Level of Inorganic Phosphorous on Laying Hen Performance and Phosphorous Balance. Major Professor: Dr. W. Guenter.

A 252-day (9 28-day periods) experiment was conducted to determine the performance of layers fed wheat-barley-soy or wheat-barley-canola diets with (.5 and .63% total phosphorous respectively) or without (.4 and .53% total phosphorous respectively) supplemental inorganic phosphorous (.1%). There were no significant (P>.05) dietary effects on egg production, feed intake, feed efficiency, egg shell quality, Haugh Unit scores, body weights or mortality either overall or within each trimester (84 days each). Body weight gain (periods 1-5, 1-9) was significantly affected (P<.05) by protein source while egg weights (periods 1-3, 1-9) and midway and final body weights tended (P<.10) to reflect protein source (always greater for soybean meal).

Three 7-day balance trials were conducted at the

beginning (IBT/Period 1), midpoint (MBT/Period 5) and end (FBT/Period 9) of the experiment to determine the phosphorous balance of the hens. Young hens were in a severe phosphorous deficiency (454 mg phosphorous/hen/day) early in the pre-trial (IBT). In the MBT and FBT phosphorous retentions were positive for both protein sources resulting in increased carcass phosphorous content over the course of the experiment. Phosphorous retentions were also positive for both levels of phosphorous supplementation but were significantly greater for the supplemented hens. The unsupplemented hens maintained carcass phosphorous reserves while the supplemented hens increased carcass phosphorous reserves. The phosphorous retentions were always greater on egg-days than on non-egg days. Estimated phosphorous availabilities ranged from 23.3% to 49.5% for soybean meal diets and 30.6% to 42.0% for canola meal diets.

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ABBREVIATIONS

AP = Available Phosphorous
BD = Basal Diet
Ca = Calcium
CM = Canola Meal
d = day
ED = Egg Day
EFPE = Endogenous Fecal Phosphorous Excreted
FBT = Final Balance Trial
h = hen
HU = Haugh Unit
IBT = Initial Balance Trial
IFPE = Ingredient Fecal Phosphorous Excreted
IP = Inorganic Phosphorous
MBT = Midway Balance Trial
MS = Mean Square
ND = Normal Diet
NED = Non-Egg Day
NPP = Non-Phytin Phosphorous
P = Phosphorous
PP = Phytin Phosphorous
RSM = Rapeseed Meal
SBM = Soybean Meal
TEP = Total Excreta Phosphorous
TP = Total Phosphorous
TPI = Total Phosphorous Intake

INTRODUCTION

Phosphorous, when added through inorganic sources, can be a costly factor in commercial layer diets. It is in the best interests of producers to lower the level of added inorganic phosphorous (IP) as much as possible while still maintaining optimum hen performance in order to maximize profitability.

Many studies have been conducted in an effort to determine dietary total phosphorous (TP) requirements of caged layers. A review of the literature reveals a wide range of dietary TP studied for maintenance of optimum egg production, egg specific gravity, egg shell thickness, Haugh Unit score and various carcass parameters. The literature review for the present study deals with reported dietary phosphorous (P) requirements and the reasons for the variability of results obtained. Effects of dietary P level on egg production, egg quality and various carcass parameters are discussed. A brief discussion on phytin phosphorous (PP) and its availability to layers is also included.

Most of the studies to date have estimated P requirement and adequacy on the basis of hen production, egg

shell quality, bone ash and bone P content. More recent researchers have included data on balance trials to aid in interpreting the variable requirement results. Dietary TP requirements determined by these methods which range from .39-.64% will be discussed.

The majority of reported studies have been carried out in the United States or eastern Canada where the dietary composition is predominantly corn-soy. In the Canadian prairies corn is not as available and is often cost-prohibitive relative to the other cereal grains, therefore corn has been replaced by wheat and/or barley in most commercial layer diets. Similarly soybean meal (SBM) can be cost-prohibitive and begs the use of alternate protein sources. Canola meal (CM), a Canadian product, has a higher P content than SBM but also higher fibre and phytate levels suggesting lower availability of the P since a higher percentage is in the form of PP. Little comparative data is available for western Canada producers regarding the availability and utilization of P in wheat-barley-soy and wheat-barley-canola diets and the requirement for supplemental IP in those diets. Therefore the present study was designed to investigate:

(a) the effect of low level IP supplementation of canola meal or soybean meal diets on laying hen performance;

(b) the P balance of layers throughout a 9-month production cycle;

(c) the performance of layers fed canola meal versus soybean meal as protein source in wheat-barley diets.

LITERATURE REVIEW

I. Dietary Phosphorous Requirement of Laying Hens to Maintain Optimum Egg Production

In the past 2 1/2 decades, many studies have been carried out in an effort to determine what level of dietary P is required for optimum egg production. The results have been varied as shown in Table 1 with the recommended dietary total phosphorous requirement ranging from .265 to .705%. As the diets used in these studies differed in basal ingredients (e.g. wheat vs. barley vs. corn) it would be beneficial to compare them expressing requirements on the basis of dietary available phosphorous (AP), thereby eliminating differences due to variable ingredient P availability. The data reported in Table 1 reveal AP requirements ranging from .16 to .45%.

Another factor to be taken into account when comparing these studies is that of feed intake which is dependent on such factors as ambient temperature, dietary energy, rate of egg production and strain of bird. To eliminate differences in feed intake between experiments, P requirements can be expressed in terms of mg P/hen/day (mg P/h/d). Table 1 shows that the recommended daily P intake of layers ranges

Table 1. Literature Survey of Dietary Phosphorous Recommendations for Laying Hens to Maintain Optimum Production.

Authors	Year	Recommended Phosphorous Levels % of Diet TP[1]	% of Diet AP[2]	mg/hen/day TP	mg/hen/day AP
Walter and Aitken	1962	.6	.36	643	386
Singsen et al.	1962	>.4	>.35	>461	>403
Crowley et al.	1963a[4]	.413	.31	434	326
	b	.385	.345	396	355
	c	.265	.225	235	200
Hinners et al.	1963	.705	--	--	--
Waldroup et al.	1967a	.54	.302	--	--
	b	.40	.162	--	--
Salman et al.	1969a	.6	--	547	--
	b	.3	--	327	--
Davidson and Boyne	1970	.55	--	609	--
Damron et al.	1974	.43	--	--	--
Reichmann and Connor	1977	.45	--	508	--
Owings et al.	1977a	.42	.19	438	198
	b	.39	.19	402	196
Garlich	1979	.28	--	350	--
Guenter	1980	.38	--	--	--
Mikaelian and Sell	1981a	.49	.26	475	252
	b	--	.16	--	155
Klingensmith and Hester	1982	.5	.3	--	--
Said and Sullivan	1982	.4	--	--	--
Waldroup and Hellwig	1982	--	.25	--	272
Klingensmith and Hester	1983	--	.3	--	--
Dilworth and Day	1983	.411	.2	--	--
Miles et al.	1983	.4	--	404	--
Junqueira et al.	1984	.6	--	517	--
Said et al.	1984a	.5	--	555	--
	b	.5	--	500	--
Roland and Farmer	1986a	.32	--	384	--
	b	.33	--	307	--
	c	.70	--	798	--
	d	.70	--	714	--
Roush et al.	1986	.54	.35	546	354
Scheideler and Sell	1986	.56-.39	.4-.2	614-405	439-202
Sell et al.	1987	.7-.46	.45-.25	711-474	457-250
		.47(32)[3]	.27(18)	485(24)	290(13)

[1] TP = Total Phosphorous
[2] AP = Available Phosphorous as determined by author
[3] Average based on the number of observations in brackets
[4] a b c d refers to trials reported within each paper.

from 235 to 798 mg TP/h/d or 155 to 457 mg AP/h/d.

The great variation in the results has been suggested to be due to such factors as strain of bird (Ingram et al., 1976; Najib and Sullivan, 1978), time of chick hatch (Bletner et al., 1977), dietary salt level (Choi et al., 1980), form of feed (Summers et al., 1976), age of bird and level of egg production (Crowley et al., 1963), stage of production (Klingensmith and Hester, 1983), proportion of organic vs. inorganic P (Waldroup et al., 1976a) and P source (Said et al., 1984).

Most of the studies reported used single phase P feeding, however more recently a step-down phase feeding of P has been investigated. The results of the two types of programs will be discussed separately, in each case highlighting the differences in experimentation contributing to the variability of recommendations.

A. Single Phase P Feeding

Owings et al. (1977) fed layer diets containing .42, .51 or .60% TP and found no differences in egg production. The basal diet was a corn-soy diet supplemented with defluorinated rock phosphate. These authors estimated AP at .19% for the low P diet (AP consisting only of non-phytin P) resulting in an intake of 198 mg AP/h/d. In a subsequent trial using similar diets but older hens for a shorter time period the AP requirement was confirmed to be .19% in part

(.09%) being supplied by IP. Hens on the .19% AP diet (.39% TP) consumed about 196 mg AP (402 mg TP) daily at production levels ranging from 64 to 74%. The authors suggested that a diet containing no IP was inadequate for maintaining egg production.

The above recommendations were somewhat lower than those reported earlier by Crowley et al. (1963). In a series of 3 studies evaluating P requirement using corn-soy diets, these authors suggested in their first trial that caged layers required no more than .41% dietary TP (.31% AP assuming 50% availability of PP) or 326 mg AP/h/d. In the second study, a dietary TP level of .385% was found to be adequate (.35% AP) which supplied 355 mg AP/h/d. In the last study they concluded, similar to Owings et al. (1977), that no more than 200-213 mg AP/h/d was required to maintain performance. The different P requirements reported in the three studies could possibly be explained by the age of the birds and the level of egg production. In the first study, the hens were on test from the 7th to the 13th months of production inclusive and produced at a rate of 65% whereas in the second study, hens were younger (first to 11th months of production inclusive) and produced at a rate of 70%. The third study employed hens during their 7th to 12th months with production ranging from 49 to 56%. The trend was for dietary AP requirement to increase (213-326-355 mg AP/h/d) as level of production increased (56-65-70%).

Other studies have recommended higher dietary P levels for laying hens. Walter and Aitken (1962) fed a wheat-soy diet containing .4% TP supplemented with dicalcium phosphate to give TP levels of .4, .5, .6 and .7% (.16, .26, .36 and .46% AP based on the assumption that 30% of the plant P and 100% of the animal and dicalcium phosphate P was available). Calcium was maintained at 1.5% with oystershell being provided free choice. Over the 308 day trial the .4% TP diet did not maximize egg production (72%) compared to the .6 and .7% diets (77% egg production) with the .5% diet yielding intermediate production (75%). It was concluded that an AP level of .36% in the diet (.6% TP) corresponding to an average daily P intake of 386 mg AP/h/d was sufficient to maintain optimum egg production (77%) while an AP level of .16% in the diet (.4% TP) corresponding to an average daily P intake of 163 mg AP/h/d was inadequate.

Junqueira et al. (1984) reported that a level of .3% TP in a corn-soy diet was inadequate for egg production relative to .6% TP in the same diet supplemented with dicalcium phosphate. Since no TP levels between .3 and .6% were tested it is not possible to conclude that .6% TP was the minimum requirement. Layers on the .6% TP diet consumed 597 and 517 mg TP/h/d in the first and second trials respectively.

Roland and Farmer (1986) investigated corn-soy diets containing a range of .3 to 1.5% TP fed to Dekalb XL hens at

48 and 60 weeks of age. Daily TP intakes of 288 and 319 mg/h did not maintain egg production relative to intakes of 714 and 810 mg/h respectively.

Most of the other studies listed in Table 1 indicated dietary TP requirements to fall between .265% (Crowley et al., 1963) and .7% (Roland and Farmer, 1986) depending on various experimental conditions and procedures employed.

Level of egg production is one factor which seems to affect the dietary P requirement. Singsen et al. (1962) concluded that the dietary TP requirement in a corn-soy diet was greater than .4% (AP level of .35%) corresponding to an average daily AP intake of 403 mg. The relatively high hen-day egg production level of 82% over this 280-day trial could be responsible for this high dietary AP requirement. Similarly, Waldroup et al. (1967) reported that corn-soy diets with .34% TP could not support even very low levels of production (50%) and required .2% supplemental IP to support a normal rate of production (74%) in one trial and .06% supplemental IP in a second study (75% production). The authors did not suggest reasons for the difference in their results. In a later study Salman et al. (1969) concluded that a corn-wheat-soy diet with .3% TP supported adequate egg production. It should be noted however that these birds in their first six months of lay were only producing at 66-68%, thus requiring less P for production.

Source of dietary P affects the dietary TP requirement.

Waldroup et al. (1967) suggested that all-plant diets (hominy feed-soy) were not capable of supporting normal egg production even though the dietary TP level was relatively high (.54%) and egg production was only 47%. In a subsequent study, these authors confirmed this earlier conclusion. Said et al. (1984) conducted a study designed to determine P requirements of two layer strains using different sources of IP. The diets were a basal corn-soy diet (.4% TP), three test diets containing supplemental dicalcium phosphate (.5, .6, .7% TP) and four test diets containing .5 and .6% TP supplemented with one of two sources of raw rock phosphate. Dicalcium phosphate being the source of supplemental P, maximum production was reached by both strains when the diet contained .5% TP (500-555 mg TP/h/d). However .6% TP was required when raw rock phosphate was the source. This suggested a greater availability of P from the dicalcium phosphate than from either of the raw rock phosphates.

Stage of production has been suggested as being, in part, responsible for variable results. Klingensmith and Hester (1983) reported a lower egg production when .3% AP was fed (vs .5 and .7% AP) for the first five months of lay with no difference in the last three months. This suggested a decrease in AP requirement in the latter stages of the laying cycle and supports the concept of phase feeding P.

B. Step-Down Phase Feeding of Dietary P

In recent years, the concept of phase feeding decreasing levels of dietary P has gained the interest of several researchers. Mikaelian and Sell (1981) fed a basal corn-soy diet (.26% AP) supplemented with dicalcium phosphate to give the following treatments: .26% AP, .36% AP, .46% AP each fed continuously; .46, .36, .26% AP and .56, .46, .36% AP fed in phases of 12, 12 and 20 weeks respectively. Percent egg production ranged from 70 to 83% and was numerically but not significantly affected by treatment. Phosphorous intake (mg AP/h/d) ranged from 252 (.26% AP treatment) to 468 (.46% AP treatment). The data from the first study suggested that continuous feeding of .26% AP was sufficient to maintain egg production. In a second study Mikaelian & Sell (1981) used similar diets where the treatments were: .16, .26, .36, .46% AP each fed continuously; .36, .26, .16% AP or .46, .36, .26% AP fed in phases of 12, 12 and 20 weeks respectively. Percent egg production ranged from 76 to 80% and was not significantly affected by treatment. Phosphorous intake (mg AP/h/d) ranged from 155 (.16% AP treatment) to 449 (.46% AP treatment). The authors concluded that continuous feeding of .16% AP was sufficient to maintain egg production. The seemingly lower levels of AP required in the second study could possibly be explained by the fact that a different strain of bird was used.

These relatively low levels of dietary P requirement were supported by Waldroup and Hellwig (1982) who reported that a dietary P level of .25% AP (272 mg AP/h/d) or phase feeding of .45% AP (19-36 weeks) followed by .25% AP (36 to 52 weeks) was sufficient to maintain egg production.

Other phase feeding studies have recommended higher dietary TP levels. Guenter (1980) fed a basal wheat-oats-soy diet (.38% TP) supplemented with varying levels of IP either continuously or in step-down phases. The treatment groups were: .78% TP fed continuous; .68, .58, .48, .38% TP fed in phases of 12 weeks each; .58% TP fed continuous; .58, .48% TP fed in phases of 24 weeks each; and the unsupplemented (.38% TP) basal diet fed continuously. Egg production was unaffected by treatment thus suggesting that a basal wheat-oats-soy diet (.38% TP) required minimum P supplementation to maintain optimum egg production. Similarly Said and Sullivan (1982) concluded that a corn-soy diet with .4% TP fed continuous was adequate for egg production.

More recently, Scheideler and Sell (1986) used 3 P treatments: .64% TP fed continuous; .56, .49, .39% TP or .64, .54, .44% TP fed in phases of 12, 16 and 20 weeks respectively. They reported no effect of diet on egg production. The average daily TP intakes were 676 mg for the first treatment, 614-565-405 mg for the second treatment and 656-656-547 mg for the third treatment. In a similar

study Sell et al. (1987) reported that a phase feeding program of.60, .48, .37% TP for 12, 16 and 20 weeks respectively was inadequate for egg production relative to a .70, .58, .46% TP program. Here the average daily TP intakes were 617-486-363 mg (each phase respectively) for the low P treatment and 711-610-475 mg (each phase respectively) for the high P treatment.

C. Summary

The average recommended daily TP intake is 485 mg (235 to 800 mg). Based on available P the average recommendation is .27% (16% to .36%) or 155 to 400 mg AP/h/d (290 mg on average) for maximum production. Step-down phase feeding of P was not shown to be beneficial in maintaining laying hen performance.

II. Dietary P Requirement of Laying Hens to Maintain Optimum Egg Quality

Extensive research has been carried out in an effort to determine the effects of dietary P level on egg quality. The findings have been inconclusive.

A. Effects of Dietary P Levels on Egg Specific Gravity

Egg specific gravity has often been measured to obtain an estimate of shell quality. Several researchers have reported no effects of dietary P level on egg specific

gravity. Walter and Aitken (1962) fed layers (over 308 days) a basal wheat-oats-corn-soy diet (.4% TP) supplemented with dicalcium phosphate to yield .4, .5, .6 and .7% TP diets. The average egg specific gravities were similar for all treatments for any given time but did decline as the season progressed. Very similar results were reported by Singsen et al. (1962) upon supplementing a corn-soy diet with dicalcium phosphate giving three treatment groups containing .2, .4 and .6% TP. Other researchers who have reported no effect of dietary TP level on egg specific gravity include Hunt and Chancey (1970) using TP levels of .38, .49, .60 and .72% in wheat-barley-oats-soy diets, Najib and Sullivan (1978) feeding TP levels ranging from .33 to .66% in corn-soy diets, Waldroup and Hellwig (1982) with AP levels ranging from .25 to .45% and Roland and Farmer (1986) using TP levels of .31, .58 and .70% in one trial and .31 and .70% in a second trial.

On the contrary, Holcombe et al. (1976) reported a response. In two trials four treatment groups of hens were offered a choice of two diets containing different levels of P. These treatment groups were offered: the same diet (.75% TP control); .19 and .46% TP; 1.0% and 2.43% TP; .19% and 2.43% TP. In both trials consumption of the 2.43% TP diets resulted in a reduction of egg specific gravity while the .19% vs .46% TP group maintained egg specific gravity comparable to the control group. Thus, the trend was for

higher P levels to cause a reduction in egg specific gravity and vice versa. These results were supported by Roland and Harms (1976). Feeding a .75% TP diet in the morning vs. a 1.5% TP diet in the afternoon decreased egg specific gravity in all four experiments relative to that of birds offered the .75% TP diet at both feedings. Feeding a .75% TP diet in the morning and a .33% TP diet in the afternoon increased egg specific gravity relative to that of the control birds in three of four experiments. Similar results (i.e. inverse relationship between dietary P and egg specific gravity) were observed by Reichmann and Connor (1977), Hamilton and Sibbald (1977), Miles and Harms (1982), Miles et al. (1983), and Roland and Farmer (1986).

More recently researchers have reported egg specific gravity to increase as dietary TP increased from .5 to .7% and then decrease as dietary TP increased above .7%. Roland and Farmer (1982) conducted a series of six experiments and found extremely low or high levels of dietary P (specific levels not given) adversely affected shell quality (egg specific gravity and shell weight) in some trials while in others reduced dietary P improved shell quality. Similarly Roland and Farmer (1986) reported egg specific gravity to increase and then decrease as TP levels increased from .33% to .70% to 1.5%.

Junqueira et al. (1984) conducted two trials using a basal corn-soy diet without and with dicalcium phosphate

supplementation to give TP levels of .3 and .6%. In both trials the higher TP diets yielded significantly higher egg specific gravities. These results differ from those presented previously and could suggest that a .3% TP corn-soy diet is insufficient to maintain shell quality and that the requirement lies between .3 and .6% TP. The variable responses of egg specific gravity to dietary P are tabulated in Table 2.

B. Effects of Dietary P Levels on Egg Shell Thickness

Eggshell thickness is often measured as an indication of shell quality. Salman et al. (1969) investigated the availability and utilization of plant P by layers and measured, among other parameters, shell thickness. The dietary treatments were: .3% TP (basal corn-wheat-soy diet); .6% TP (all from plant sources) and .6% TP (.3% supplemental P from dicalcium phosphate). There was no effect of dietary treatment on shell thickness or egg shell formation. Davidson and Boyne (1970) fed barley-oats-wheat-fish diets with bone flour providing extra P to give TP levels of .54, .73 and .94%. There was no effect of treatment on shell thickness. Similar results have been supported by Hinners et al. (1963), Mikaelian and Sell (1979), Hamilton (1980), West et al. (1980), Scheideler and Sell (1986) and Sell et al. (1987).

To the contrary, several researchers have reported that

Table 2. Literature Survey of the Effects of Dietary Phosphorous Levels on Egg Specific Gravity.

Authors	Year	Dietary TP[1] Levels (%)	Effect of Increasing Dietary ATP on Egg Specific Gravity
Walter and Aitken	1962	.4,.5,.6,.7	none
Singsen et al.	1962	.2,.4,.6	none
Hunt and Chancey	1970	.38,.49,.60,.72	none
Najib and Sullivan	1978	.33-.66	none
Waldroup and Hellwig	1982	.25-.45 (AP)[2]	none
Roland and Farmer	1986a[3]	.31,.58,.70	none
	b	.31,.70	none
Holcombe et al.	1976	.19,.46,.75,1.0,2.43	decrease
Roland and Harms	1976	.33,.75,1.5	decrease
Reichmann and Connor	1977	.45-1.42	decrease
Hamilton and Sibbald	1977	.54,.59,.64,.69	decrease
Goto et al.	1978	.42,.52,.60 (AP)	decrease
Miles and Harms	1982	.42,1.4	decrease
Miles et al.	1983	.17-2.3	decrease
Roland and Farmer	1986	.32,.7,1.5	decrease
Roland and Farmer	1982	.33,.7,1.5	quadratic
Junqueira et al.	1984	.3,.6	increase

[1] TP = Total Phosphorous
[2] AP = Available Phosphorous
[3] a b refers to trials reported within the paper.

dietary P level does have an effect on shell thickness or strength. Taylor (1965) fed a basal corn-wheat-soy diet with .46% TP (.23% PP) and an experimental diet of 1.0 TP. The birds were fed the two diets alternately over four 2-week periods and shell thickness was found to significantly decrease as dietary TP level increased and vice versa. These results have been confirmed by Said et al. (1982), Angulo et al. (1982), Zumbado and Britton (1983) and Daghir and Farran (1983). The variable responses of egg shell thickness to dietary P level are tabulated in Table 3.

C. Effects of Dietary P Levels on Haugh Unit Scores

Interior quality measured as Haugh units (HU) in general has not been affected by dietary TP. Hamilton and Sibbald (1977) fed corn-wheat-soy diets with .58, .55, .50 or .45% AP from 20 to 71 weeks of age with HU being measured on days 165, 225, 277, 335, 390, 445 and 490. There were no significant effects of P level on HU except at day 165 which was an unexplained result. It would thus seem that a dietary AP level of .45% (.6% TP) is sufficient to maintain interior egg quality. In a subsequent trial by Hamilton (1980) force-molted layers were fed mixed cereal diets containing .49 and .79% TP (.34 and .6% AP) from 646 to 730 days of age (92.3 to 104.3 weeks of age). Haugh Units were measured from 727-730 days of age with no significant differences being observed due to dietary P level. It would

Table 3. Literature Survey of the Effects of Dietary Phosphorous Levels on Egg Shell Thickness or Strength.

Authors	Year	Dietary TP[1] Levels (%)	Effect of Increasing Dietary TP on Egg Shell Thickness
Hinners et al.	1963	.705-1.018	none
Salman et al.	1969	.3,.6	none
Davidson and Boyne	1970	.54,.73,.94	none
Mikaelian and Sell	1979	.26,.36,.46,.6(AP)[2]	none
Hamilton	1980	.34,.6 (AP)	none
West et al.	1980	.16,.36	none
Scheideler and Sell	1986	.56-.39	none
Sell et al.	1987	.7-.46	none
Taylor	1965	.46,1.0	decrease
Said et al.	1982	.4,.5,.6.,.7	decrease
Angulo et al.	1982	.35,.5 (AP)	decrease
Zumbado and Britton	1983	.3,.6,1.2 (AP)	decrease
Daghir and Farran	1983	.15,.25,.35 (AP)	decrease

[1] TP = Total Phosphorous
[2] AP = Available Phosphorous

therefore appear that .34% AP (.49% TP) is sufficient to maintain HU. These results have been supported by Daghir and Farran (1983).

Several researchers have reported an effect of dietary P level on HU score but the findings have been contradictory. Said et al. (1984) conducted two trials with layers being fed different P sources and levels. The first used layers 26-68 weeks old while the second used the same birds at 76-116 weeks of age (after force-molting). Dietary treatments consisted of a basal corn-soy diet (.4% TP) supplemented with dicalcium phosphate to give TP levels of .51, .60 and .70% or supplemented with two sources of raw rock phosphate to give TP levels of .5 and .6% on each source. These treatments were the same for both trials. In the first trial as TP level increased due to supplementation with dicalcium phosphate or the second source of raw rock phosphate HU scores increased linearly. Conversely as TP level increased due to supplementation with the first source of raw rock phosphate HU scores decreased linearly. It was suggested that trace minerals such as vanadium in this source of IP may have caused the result by decreasing albumen quality. There were significant differences in terms of HU between the two sources of raw rock phosphate and dicalcium phosphate. Similar results were obtained with the older hens except the differences between raw rock phosphate and dicalcium phosphate did not exist. However, a

quadratic response of HU to TP level when supplemented with dicalcium phosphate was reported. The results of trials measuring the response of HU to dietary P levels are tabulated in Table 4.

D. Summary

Dietary P level has had variable effects on egg specific gravity (Table 2), eggshell thickness (Table 3) and HU score (Table 4). Some studies (.2 to .72% TP) have indicated no effect of P on egg specific gravity while others (.17 to 2.43% TP) have shown an inverse relationship between dietary TP and egg specific gravity. Egg specific gravity has also responded to dietary TP quadratically (.33 - .7 - 1.5%) and linearly (.3-.6). Some trials (.16 to 1.018% TP) have indicated no effect of P on egg-shell thickness or strength while others (.15% AP to 1.0% TP) have shown a linear relationship between dietary P and eggshell thickness. In general HU scores have not been affected by dietary P level (.15%AP to .79%TP) but raw rock phosphate caused linear and inverse relationships between HU and dietary P level in two separate trials which was attributed to high levels of vanadium in these sources.

Table 4. Literature Survey of the Effects of Dietary Phosphorous Levels on Haugh Unit Scores.

Authors	Year	Dietary TP[1] Levels (%)	Effect of Increasing Dietary TP on Haugh Unit Scores
Hamilton & Sibbald	1977	.45,.5,.55, .58 (AP)[2]	none
Hamilton	1980	.49,.79	none
Daghir and Farran	1983	.15,.25,.35 (AP)	none
Said et al.	1984a[5]	.4,.5,.6,.7	increase[3]
	b	.4,.5,.6,.7	decrease[4]

1 TP = Total Phosphorous
2 AP = Available Phosphorous
3 Sources of IP were dicalcium phosphate and a second source of raw rock phosphate
4 Source of IP was a first source of raw rock phosphate
5 a b refers to trials reported within the paper.

III. Effects of Dietary P Levels on Carcass Parameters

Researchers, in addition to records on egg production and egg quality, have measured various carcass parameters in an effort to determine adequacy of diets in terms of P level. Presumably a P deficiency would be reflected in some of the measurement values obtained, however as for egg production and egg quality, results have been inconsistent. Reports showing no carcass response and those showing a response to dietary P are discussed separately.

A. Reports Suggesting No Carcass Response to Dietary P

Results of the trials finding no effect of dietary P level on carcass parameters are tabulated in Table 5.

Salman et al. (1969) fed layers different sources and levels of P for six 4-week periods. The treatments were: basal corn-wheat-soy diet containing .3% TP (all from plant sources); .6%TP (all from plant sources) and .6% TP (.3% from plant sources and .3% IP). Analysis of tibia bone ash revealed no differences due to dietary P level or source indicating equal availability of plant and inorganic P to the layers for bone maintenance.

Similar TP levels (.3%, .39%, .48%, .57%) were employed by Owings et al. (1977) in corn-soy diets supplemented with defluorinated rock phosphate. Data indicated no significant difference in femur ash due to dietary P level although

Table 5. Literature Survey of Research Which has found No Effect of Dietary Phosphorous Level on Various Carcass Parameters.

Authors	Year	Dietary TP[1] Levels (%)	Carcass Parameters Measured
Salman et al.	1969	.3,.6	tibia ash (%)
Owings et al.	1977	.3,.39,.48,.57	femur ash (%)
Mikaelian and Sell	1979	.26,.36,.46 (AP)[2] .46-.36-.26 (AP) .6-.46-.36 (AP)	tibia ash (%) tibia P (%)
Mikaelian and Sell	1981a[3]	.26,.36,.46,.56 (AP) .46-.36-.26 (AP) .56-.46-.36 (AP)	tibia weight (g) tibia ash (%) tibia P (%) carcass ash (%) carcass P (%)
	b	.16,.26,.36,.46 (AP) .36-.26-.16 (AP) .46-.36-.26 (AP)	carcass ash (%) carcass P (%)
Edwards and Suso	1981	.45,.55,.65,.75	tibia ash (%) pygostyle & coccygeal vertebrae ash (%)
Sell et al.	1987	.6-.48-.37 .7-.58-.46	carcass ash (%) carcass P (%)

1 TP = Total Phosphorous
2 AP = Available Phosphorous
3 a b refers to trials reported within the paper.

numerically the basal diet (.3% TP) resulted in the lowest femur ash content while the .48% TP diet had the highest level. It is possible that due to the brevity of the trial (8 weeks) the femur ash content had not yet responded to dietary P levels.

Mikaelian and Sell (1979, 1981) in a series of three trials fed AP levels ranging from .16 to .6% either continuously or in stepdown phase feeding programs. The diets were corn-soy with varying levels of dicalcium phosphate and the trials lasted 42 to 44 weeks each. Tibia and carcass dry weight, % ash and %P in ash were not responsive to dietary AP level but the % carcass ash increased numerically with increasing levels of dietary AP. At the end of treatment 1 (relative to its start) tibias were larger with a higher % ash and a greater quantity of P per tibia while %P in the bone ash, % carcass ash, %P in the carcass ash and total quantity of P in the carcass decreased irrespective of diet. The data in these trials suggest that relatively low dietary AP levels will maintain the health of layers over prolonged feeding periods.

Other researchers finding no effect of dietary TP level on carcass parameters include Edwards and Suso (1981) with dietary TP ranging from .45 to .75% and Sell et al. (1987) phase feeding TP levels ranging from .37 to .70%.

B. Reports Suggesting Carcass Response to Dietary P

Results of the trials finding an effect of dietary TP level on various carcass parameters are tabulated in Table 6.

In an early trial Singsen et al. (1962) fed corn-soy diets supplemented with dicalcium phosphate to give dietary TP treatments of .2, .4 and .6%. It was observed that birds fed the .2% TP had a lower % tibia ash than those fed .4 or .6% TP 12, 24, and 40 weeks into the trial. Generally there was a numerical trend where increasing dietary TP increased tibia ash %.

Similar diets (.18, .23, .43% AP) were fed by Garlich et al. (1982) in a study to evaluate the femurs of laying hens from 10-16 months of age. These authors reported that dietary P level had no significant effects on dry, fat-free femur weight but that femur ash (%) increased numerically as dietary AP level increased, and was significantly higher for hens fed the .43% AP compared to the other two groups. Further comparisons indicated that femurs of laying hens fed P-deficient diets had a reduction in mineral content but not in organic matter.

Said et al.(1984) in two trials fed basal corn-soy diets (.4% TP) supplemented with dicalcium phosphate to give TP levels of .5, .6 and .7%; or supplemented with one of two sources of raw rock phosphate to give TP levels of .5 and .6% for each source. Birds receiving the basal diet had a

Table 6. Literature Survey of Research which has found an Effect of Dietary Phosphorous Level on Various Carcass Parameters.

Authors	Year	Dietary TP[1] Levels (%)	Carcass Parameters Measured
Singsen et al.	1962	.2,.4,.6	tibia ash (%)
Garlich et al.	1975	.3,.64 .3-.64	femur density
Garlich	1979	.23,.28,.48 (AP)[2]	femur density
Garlich et al.	1982	.18,.23,.43 (AP)	femur weight (%) femur ash (%)
Said and Sullivan	1982	.4,.45,.5,.55,.6 .6-.55-.5-.45 .55-.5-.45-.4	tibia ash (%) tibia breaking strength
Said et al.	1984	.5,.6,.7	tibia ash (%)
Bar and Hurwitz	1984	.22,.67 .67-.33	tibia ash (%)
Scheideler and Sell	1986	.64 .56-.49-.39 .64-.54-.44	femur ash (g)

[1] TP = Total Phosphorous
[2] AP = Available Phosphorous.

significantly lower % bone ash indicating the marginal TP level in this diet. As dietary TP level increased there was a numerical (but not significant) increase in bone ash %. It was noted that there was a significant effect of source of P on tibia ash % with dicalcium phosphate giving higher bone ash % than the raw rock phosphate sources.

Said and Sullivan (1982) fed layers continuously or in step-down phases dietary TP levels ranging from .4 to .6% in corn-soy diets over twelve 28-day periods. Dietary TP level had significant linear and quadratic effects on bone ash at 68 weeks of age and linear effects on tibia breaking strength at 44, 56 and 68 weeks of age. Phase feeding P significantly enhanced % bone ash at 68 weeks of age over the continuous feeding of P. These results were supported by Scheideler and Sell (1986) in a study phase feeding dietary TP levels ranging from .39 to .64%. The response was for femur ash to decrease as dietary TP level decreased indicating an insufficient supply of dietary P to prevent bone demineralization.

The ability of bones to serve as a reservoir of P over short-term P deficiencies was studied by Garlich et al. (1975). The basal diet (BD) was a corn-soy diet with .3% TP and a normal diet (ND) was obtained by supplementation with inorganic P to give a TP level of .64%. Group 1 was fed the ND for 21 days; Group 2 was fed the BD for 6 days followed by the ND for 15 days; Group 3 was fed the BD for 9 days

followed by the ND for 12 days; and group 4 was fed the BD for 21 days. Femur density showed a significant decrease in those hens fed the BD for 21 days compared to those fed the BD for 0 or 9 days only. Thus layers were able to consume a P-deficient diet (by NRC standards) for up to 9 days without any detrimental effects. This suggested that the bone can indeed serve as a true reserve of P which can be released for productive use by the hen and replenished. In a second trial, Garlich (1979) investigated the extent of bone P reserves using three dietary treatments (.23, .28, .48% AP) fed from 40-64 weeks of age. Femur density significantly increased with each level of dietary AP. At the end of the trial, when all hens were fed a diet with only .18% AP, the bone reserves supported good egg production for 1, 2 and 3 weeks respectively. The authors concluded that laying hens should consume at least 350mg AP/h/d to maintain adequate bone mineral reserves. Finally, Bar and Hurwitz (1984) investigated the effect of P deficiency on medullary bone ash of 8-month-old layers fed a P sufficient (.67% TP) or deficient (.22% TP) diet. They reported that ash content of the structural segment of the tibia tended to be reduced by P deficiency while total ash content of the tibia medullary segment was significantly reduced by dietary P deficiency. It was suggested that since no known mechanism exists for the active removal of P from the bone during P deficiency and hypophosphatemia, the reduction in medullary bone ash by

P deficiency appears to result from inhibition of bone formation.

C. Summary

The effects of dietary P levels on various carcass parameters (tibia or femur ash, % P in bone ash, carcass ash, bone density, bone weight, bone breaking strength, carcass P) are just as variable as the performance and egg quality data. The literature surveys (Tables 5 and 6) show that .2 - .75% TP levels in diets of laying hens have been investigated. Other factors (eg. egg production level) in addition to absolute P levels affect P utilization and these need to be identified and nullified between trials to allow for accurate comparisons.

IV. Phytin P in Layer Diets

A. Phytin P

Much of the P present in poultry feeds (grain and protein sources) is in the form of phytin phosphorous which is poorly available. Several reviews have been written about PP including reviews by Oberleas (1973), Nelson (1980) and Maga (1982) from which the following information is supplied.

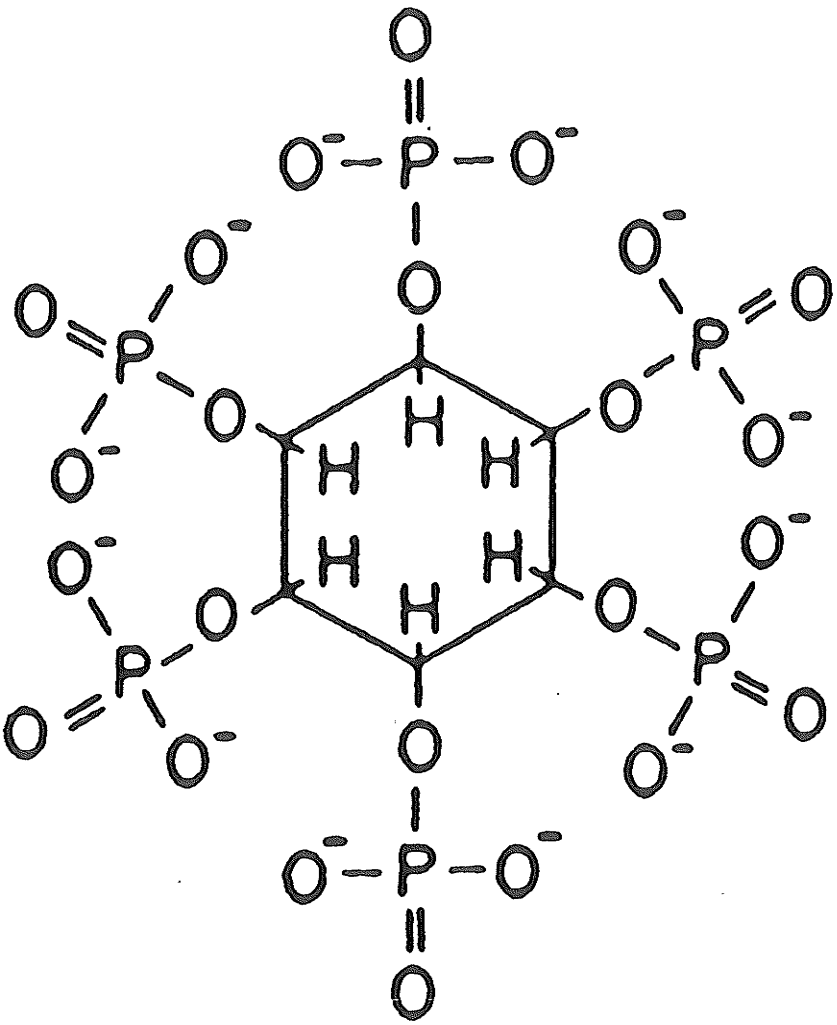
Phytin was first encountered as early as 1872 by Pfeffer. Phytate, phytates, phytic acid and phytin are all

terms referring to various polyphosphorylated inositols found in nature. Phytic acid is also known as myo-inositol hexaphosphoric acid or 1,2,3,4,5,6-hexakis (dihydrogen phosphate). Myoinositol and its structure is given in Figure 1. Nine sterisometric inositols are possible but only the myo-form has been isolated from plants. The phytin refers to the calcium-magnesium salt of phytic acid while phytate refers to the mono to dodeca anion of phytic acid.

Phytate is a strong acid that forms a wide variety of salts with heavy metals, depending on the pH and presence of secondary cations. The synergistic effect of two or more cations increases the precipitation of phytate as salt, especially zinc-calcium and zinc-copper. Phytic acid and its derivatives thus bind essential dietary minerals making them unavailable or only partially available for absorption. The most abundant cations found in phytate are Mg and K while Ca, Cu, Mn, Fe and Zn are found to a lesser degree. At a pH of 7.4 phytate complexes with metals in the following decreasing order: $Cu^{++} > Zn^{++} > Co^{++} > Mn^{++} > Fe^{++} > Ca^{++}$. Research data has indicated decreased availability of such minerals as Ca, P, Zn, Fe, Mn, Cu and Mg in the monogastric due to the presence of phytate.

In order for the PP to be utilized by animals the phytate must be hydrolyzed by an enzyme, phytase, to yield phosphoric acid and/or its salts. This enzyme is present in some feed ingredients and has some activity (eg. rye, wheat,

Figure 1. Structure of Phytic Acid
(myoinositol 1, 2, 3, 4, 5, 6 - hexakis dihydrogen phosphate
(Scott, Nesheim and Young, 1982)



barley) but is absent in other feed ingredients (eg. corn, soybean meal). The phytase present in the mature seeds appears to have little effect on the phytate in dry or dormant seeds. Under conditions of elevated temperature and moisture (eg. storage conditions) activity seems to increase but further increases in temperature and moisture (eg. steam pelleting) tend to decrease or completely inactivate the enzyme. Intestinal phytase does not seem to play a part in phytate hydrolysis in poultry.

Several researchers have investigated the PP content (% of TP) in ingredients of plant origin. Nelson et al. (1968) reported on corn, wheat, barley and soybean meal. O'Dell et al. (1972) presented data for wheat and corn and Lolas et al. (1976) reported on several varieties of wheat, barley and soybean meal. In summary these reports indicated that the PP content as a % of TP ranges from 62 to 80% in wheat; 66 to 84% in corn; 56 to 70% in barley and 51 to 58% in soybean meal, these being the major ingredients in layer feeds.

Since PP is a substantial component of the TP, much research has been done to determine the actual utilization of PP by poultry. Nelson (1967) in his review suggested that most scientists used the rule of thumb that 1/3 of P in plant material was IP and available to monogastric animals. This was based on the 1960 NRC recommendation by the Committee on Animal Nutrition that approximately 30% of

plant P could be considered available. However Nelson (1967) reported wide disagreement between investigators on the ability of poultry to utilize PP. The discrepancies were mainly due to factors such as source of PP, criteria of response measured, age of poultry, dietary calcium levels, dietary vitamin D levels, etc. The author's conclusions were as follows: a) in order to be utilized, PP must be hydrolyzed to yield IP; b) the chick absorbs and utilizes dietary P without regard to its organic or inorganic origin; c) young birds have a limited ability to hydrolyze PP, however this ability increases with age up to maturity; d) Ca at the levels required by poultry has an adverse effect on the utilization of PP; e) vitamin D3 probably enhances the utilization of PP by animals to a limited extent; f) phytase enzyme, present in certain feed ingredients and possibly secreted by the intestine, hydrolyzes PP to a form that can be utilized; and g) the NAS-NRC recommendation that 30% of the plant TP is available to animals can usually be applied to a mixture of ingredients but not to individual ingredients.

B. Utilization and Retention of Phytin P by Laying Hens

Many studies have been carried out in an effort to determine the utilization and retention of PP by laying hens. A review of the factors affecting P availability to laying hens was published by Peeler (1972). The factors

which affect utilization of P are as follows: type of diet fed, chemical form of P, Ca:P ratio, age of bird, dietary fat and energy levels, plane of nutrition, environment, hormones, disease, parasites, protein levels, trace element levels, interactions with other minerals and nutrient chelating agents, physical nature of the P source and other feedstuffs in the diet (especially particle size) and feed processing. Peeler reported P availabilities ranging from 0-80% when using mortality, egg production and bone mineral changes as response criteria. He also suggested that the biological availability of PP depended on the dietary Ca level, level of egg production and management conditions.

Nott et al. (1967) suggested that % recovery of ingested PP in the droppings of layers was 61, 84, 94 and 95% when fed diets with 1.5, 2.5, 3.5 and 4.5% Ca respectively. They concluded that when the hens were fed levels of Ca which were adequate for optimum shell calcification they were unable to utilize any of their dietary PP. The authors proposed that the P requirements of poultry should be expressed as non-phytin P (NPP) (available phosphorous).

Low levels of PP utilization were also reported by Nelson (1976) using the chromic oxide comparative balance method to determine the amount of natural PP hydrolyzed by laying hens (Single-Comb White Leghorns 6 months in lay). The two experimental diets were corn-soy (.217%PP) and

corn-wheat-soy (.322%PP) where wheat replaced 1/2 of the corn. Hydrolysis of PP was 8% and 13% for the corn and wheat-corn diets respectively, with hens showing little improvement over chicks in their ability to hydrolyze phytate in corn diets. These rates are at the low end of the scale presented by Peeler (1972) possibly due to the variety of wheat/corn or a more acid pH in the intestine which could reduce phytase activity. In vitro hydrolysis of phytate did suggest the presence of phytase in both wheat and corn with wheat having the greatest activity.

Much higher availabilities of PP were reported by Das and Netke (1979) using basal corn-groundnut diets containing .53% TP (.155% PP and .376% NPP). Two trials were conducted, one using variable levels of supplemental dicalcium phosphate and the other comparing dicalcium phosphate to raw rock phosphate supplementation. Results of the first trial indicated PP retentions of 56.1 to 65.1% and NPP retentions of 23.0 to 39.6%. In the second trial PP retentions were 47.6 to 61.5% and NPP retentions were 11.3 to 30.4%. The PP retentions are higher than the 30% suggested by NAS-NRC (1960) possibly due to the variety of maize and groundnut used. The low availability of NPP (lower than PP) could be due to the fact that the level of TP used was above the required level.

Retention of plant P has been investigated in a balance trial by Choi et al. (1979) studying the P excretion pattern

of laying hens in relation to the egg cycle. Hens were fed a basal corn-soy diet of .3% TP all from plant sources. Control birds had an average daily intake of 302.6mg TP and an average daily excretion of 278.3mg TP for a 24-hour retention of 24.3mg. If an egg contains 136mg TP then the hens had to withdraw more than 100mg TP from their body reserves on those days when an egg was laid. Excretion of P (%) increased with time following oviposition, and was 93.3% over the 24-hour period. During the first four hours after oviposition, eggshell deposition is negligible and active redeposition of bone minerals is occurring. Thus serum P is at its lowest level, hens are physiologically in maximum need of P and endogenous excretion of P should be at its minimum. These researchers found an apparent digestibility value of 28.4% for the plant P over these first four hours post-oviposition which is not an unreasonable level. As shell calcification progressed, (4-8, 8-12, 12-24 hours post-oviposition) % P excretion was 82.4, 93.2, and 104.0% respectively. These results reflect the fact that shell calcification occurs more actively during the period of 4-24 hours post-oviposition resulting in increasing endogenous P excretion. These researchers concluded that the maximum apparent digestibility of the dietary plant P (corn-soy) was 28.9% during the first four hours post-oviposition when the endogenous excretion of P was theoretically minimized, and that hens had to withdraw approximately 100mg of TP from

their body reserves to produce an egg when .3% TP was in the diet.

Phosphorous balance studies have also been carried out in an effort to determine P utilization. Mikaelian and Sell (1980) fed hens one of four dietary treatments (.39, .49, .59, and .69% TP). Hens consumed an average of 325, 384, 481 and 579 mg TP daily and excreted an average of 284, 361, 415, and 519 mg TP daily for absolute daily retentions of 41, 23, 66 and 60 mg TP (excluding the TP deposited in laid eggs) for the four dietary treatments respectively over a period of six days. When the P content of the eggs was included the hens were in a daily negative TP balance of 84, 100, 60 and 66 mg for the four treatments respectively.

From the same laboratory, Scheideler and Sell (1986) reported a balance trial of hens fed one of three dietary treatments: .64%TP fed continuous; .56, .49, .39% TP and .64, .54, .44% TP fed in phases of 12, 16, and 20 weeks each. Total P retentions were measured at 34, 42, 50, 62, and 72 weeks of age (excluding egg TP content). The average retentions were 135, 149, and 157mg TP/h/d for the three treatments. Generally the hens were in a negative P balance at weeks 42, 62 and 72 but in a positive P balance at weeks 34 and 50 when considering an estimate of 100 mg TP in the eggs.

C. Availability of PP from Canola Meal and Soybean Meal for

Poultry

Canola meal is a readily available, inexpensive (relative to SBM) protein source used throughout Western Canada in poultry diets. Canola Meal has a considerably higher P content than SBM (1.25 vs. .87%) but also higher fiber (12.0 vs. 6.5%) and phytic acid (1.92 vs. 0.85%) levels. Consequently a higher percentage of the P exists as PP (.54 vs. .24%) (Nwokolo and Bragg, 1977). These authors also demonstrated a significant inverse relationship between P availability and crude fiber (r= -0.91) or phytic acid (r= -0.93) content of feed ingredients (palm kernel, soybean, cottonseed and rapeseed meals). Research has thus been carried out to determine the availability of this PP.

Nwokolo et al. (1976), using four-week-old broiler chicks, determined the P availability of rapeseed meal (RSM) (1.25% P) and SBM (.87%P) to be 74.8 and 89.3% respectively. This was calculated as:

$$\frac{TPI - (IFPE - EFPE)}{TPI} \times 100$$

where TPI = total P intake; ITPE = ingredient fecal P excreted; and ETPE = endogenous fecal P excreted (purified diet excreta). Thus calculated AP levels for RSM and SBM were .94 and .78% respectively. This relatively high P availability was supported by the findings of Nwokolo and Bragg (1980) testing seven samples of RSM from three different cultivars of rapeseed on three-week old broiler chicks. Total P content averaged 1.22% (1.2 - 1.25%) with P

availability averaging 75.3% (65.0-81.0%) giving a calculated AP level of .92% (.78 - 1.01%) in RSM.

Similar P availabilities were reported by Guenter and Ward (1986) feeding CM (1.16%P), SBM (.8%P) or a 50/50 blend of CM and SBM (.91%P) to four-week-old broilers. Phosphorous availabilities were determined to be 67.7, 84.9, and 74.9% respectively with calculated AP levels of .79, .68, and .68% respectively. In a further trial these authors fed broilers a wheat-soy diet (.7% AP) with CM and fishmeal substituting 0, 33, 67, and 100% of the SBM. During the second week, P retentions, (measured as:

$$\frac{TPI - TEP}{TPI} \times 100$$, where TPI = total P intake; and TEP = total excreta P),

were 53.2, 52.1, 55.6 and 42.6% respectively for the four diets (average 50.9%) while during the third week the P retentions were 45.4, 46.6, 43.4 and 37.0% respectively (average of 43.1%). The P retention calculation does not account for endogenous P excretion so the endogenous portion of the excreta P is assumed not to be retained resulting in lower retention values relative to the calculated availability values where the endogenous portion of the excreta P is assumed to have been available. These authors concluded that the mineral availability in CM generally is lower than in SBM, probably due to the higher levels of fiber and/or phytate, and that mineral retention in broilers was significantly reduced when CM was fed at a level above

20% in the diet.

March (1987) studied growth and bone mineralization in White Leghorn chicks fed diets containing 30% CM supplemented with different levels of IP. Bone mineralization was poor in the chicks fed the diet containing only .029% of IP and increasing the IP supplement to .295% of the diet resulted in maximum mineralization. It was thus concluded that CM, fed at levels up to 30% in the diet of White Leghorn chicks, will allow normal bone mineralization when the diet is supplemented with 0.3% of IP.

The presented availability data indicate that:

a) the availability of P from CM is higher than traditionally thought, and is marginally lower than that of SBM.

b) if it holds true that mature layers can better utilize PP than can chicks, then one would expect PP to be even more available than the 65 - 81% reported here since three- to four-week-old chicks were used in these trials.

c) CM, in spite of its high crude fiber and phytic acid levels, is potentially a better source of P for poultry than SBM due to its high TP content and relatively high PP availability resulting in AP levels higher than SBM.

D. Summary

Much of the plant P (50-60% of wheat, barley, corn or soybean meal P) is present as PP. This phytate must be hydrolyzed by phytase enzyme (present in feed ingredients or derived from intestinal microflora) to yield myo-inositol and ortho-phosphoric acid or its salts before the P can be utilized. Utilization of PP by laying hens varies greatly between experiments (0-80%). In general, balance trials have indicated negative P balances but there are so many variables affecting utilization of PP by layers that it is difficult to determine exactly what level of dietary P is required to maintain optimum production and egg quality.

V. Experimental Objectives

Because of the inconsistency of results published in the past regarding dietary P requirement for layers and because most studies were conducted with corn-soy or wheat-soy diets, the objectives for the current study were as follows:

1. To determine the effect of low level supplementation of dicalcium phosphate in wheat-soy and wheat-canola diets on production.

2. To determine the effect of low dietary P feeding on P balance and P status of laying hens throughout a 9-month production trial.

3. To determine the performance of laying hens fed a

wheat-barley based diet supplemented with either soybean meal or canola meal as protein source.

MATERIALS AND METHODS

I. General Experimental Information

A. Housing Conditions

Three hundred and twenty commercial Single Comb White Leghorn layers (Shaver 288) were housed in 2-tier wire cages (40.6 x 40.6 cm) in an environmentally controlled layer barn at the experimental poultry facilities, Department of Animal Science, Faculty of Agriculture, University of Manitoba. The birds were randomly distributed on 2 tiers of cages at a stocking density of 4 birds/cage, 2 cages/replicate allowing each bird 413 cm^2 of floor space. The cages were equipped with a wide mouth trough feeder and 1 Hart cup/2 cages. Feed and water were provided ad libitum. Barn temperatures were maintained at 18-20^oC and the lighting program was 15h of continuous light/day followed by 9h of darkness.

B. Experimental Design and Start-Up

The pullets were housed in the layer barn at 20 weeks of age. Until 1 week before the initiation of the trial the hens were fed a standard layer diet (Table 7). One week before the start of the trial hens were fed 1 of 4

Table 7. Ingredient Composition of the Pre-Test Layer Diet and the 4 Experimental Diets (fed for 9 28-Day Periods).

Ingredient	Pre-Test Layer Diet	Experimental Diets #1	#2	#3	#4
			%		
Wheat	--	45.0	45.0	45.0	45.0
Barley	69.0	19.4	19.2	16.9	16.7
Soybean Meal (47 1/2%)	18.3	17.6	17.6	1.1	1.1
Canola Meal	--	--	--	20.0	20.0
Alpha-Cel	--	1.0	1.0	--	--
Alfalfa	1.0	2.0	2.0	2.0	2.0
Calcium Carbonate	4.3	6.0	5.7	6.0	5.7
Oystershell	2.0	3.0	3.0	3.0	3.0
Poultry Grit	.5	--	--	--	--
Biophos	1.7	--	.5	--	.5
Tallow	1.7	3.5	3.5	3.5	3.5
Sunflower Oil	--	1.0	1.0	1.0	1.0
Vitamin Premix[1]	1.0	1.0	1.0	1.0	1.0
Mineral Premix[2]	.5	.5	.5	.5	.5
	100.0	100.0	100.0	100.0	100.0

[1] Vitamin Premix supplied the following per kg of diet: Vitamin A, 8250 iu; Vitamin D_3, 1000 iu; Vitamin E, 5.46 iu; Vitamin B_{12}, 11.2 ug; Niacin, 6.6 mg; Calcium Pantothenate, 4.4 mg; Riboflavin, 2.2 mg; Choline Chloride, 110 mg; Methionine, 490 mg.

[2] Mineral Premix supplied the following per kg of diet: Manganese, 104 mg; Zinc, 37 mg; Sodium, 1.9 g; Chloride, 2.9 g.

experimental diets (Table 7) to get them accustomed to the feed. These diets were randomly assigned to each experimental unit (10 replicates/treatment, 8 birds/replicate). At 27 weeks of age the trial started with production and performance data being gathered. Hens were kept on trial over 9 28-day periods until 63 weeks of age.

C. Experimental Procedures

1. Dietary Treatment Preparation. The 4 experimental diets (Table 7) were formulated to be balanced as closely as possible for energy, protein, fiber, calcium and linoleic acid using ingredient nutrient content published by Scott et al., 1982. However 2000 kcal/kg was used as an energy value for CM. The differences were in terms of P content and protein source. Calculated and determined values for energy, protein, fiber, calcium, phosphorous, and linoleic acid of each diet are presented in Table 8.

Feed was mixed in a vertical screw-type mixer (550kg capacity) on 7 occasions. Each time (except once) the order of mixing was diet #1, #3, #4 and #2 to avoid any possible cross-contamination of control diets with biophos. All ingredients were carefully weighed out and added in the order in which they are listed in Table 7. After 5-10 minutes of mixing a sample was drawn of each individual diet and kept for analysis.

Table 8. Calculated Nutrient Content and Chemical Analysis of the Pre-Test Layer Diet and the 4 Experimental Diets (fed for 9 28-Day Periods).

	Calculated Nutrient Content				
	Pre-Test	Experimental Diets			
Ingredient	Layer Diet	#1	#2	#3	#4
ME[1] (MJ/kg)	10.38	11.75	11.73	11.39	11.37
Protein (%)	16.2	16.2	16.2	16.0	16.0
Fiber (%)	4.9	4.6	4.6	4.7	4.7
Total Calcium (%)	2.78	3.53	3.52	3.62	3.61
Total P (%)	.73	.36	.46	.47	.58
Available P[2] (%)	.54	.18	.28	.24	.34
Linoleic Acid (%)	.71	1.08	1.08	1.06	1.06

[1] ME = Metabolizable Energy
[2] Calculated on the basis of 50% of plant P + 100% of supplemental P

	Chemical Analysis				
	Pre-Test	Experimental Diets			
Ingredient	Layer Diet	#1	#2	#3	#4
Protein (%)	16.1	16.7	16.2	16.9	17.3
Total Calcium (%)	2.86	3.50	3.36	3.46	3.38
Total P (%)	.80	.40	.50	.53	.63

2. Egg, Feed, Body Weight and Mortality Records. Eggs were gathered by hand twice daily (10:30am and 3:30pm) and recorded for each replicate. All cracked and shell-less eggs were recorded as they occurred.

Feed was weighed into the feeders twice per week and weighed back at the close of each of the 9 periods. The feeders were not overfilled so as to minimize feed wastage. Feed efficiency (g feed/g eggs) was calculated for each replicate for each 28-day period.

Individual live body weights were recorded and averaged for each replicate at the start (Period 1), midpoint (Period 5), and end (Period 9) of the trial.

All mortalities were recorded as they occurred and the birds weighed. Dead birds were submitted for autopsy to a poultry pathologist at the Provincial Veterinary Laboratory, Manitoba Department of Agriculture.

3. Egg Quality Procedures. Egg quality was measured on 6 occasions. These were at the start of the trial (Period 1) and at the end of the trial (Period 9), as well as on days 6 and 7 of periods 2, 4, 6, and 8. Eggs were tested over a period of 2 days at each sampling time. Each day a maximum of 5 eggs/replicate was collected for analysis. If more than 5 eggs/replicate were laid on 1 day then the first 5 sound eggs were used. If less than 5 sound eggs were

available then either more than 5 were used on the second day to make up the balance of 10 or analysis was completed on only those sound eggs available.

Upon collection eggs were identified by replicate and number and three measurements were recorded for shell elasticity (deformation) using a Marius Deformation Apparatus (Marius Instrumentation, Utrecht, Netherlands). Eggs were then stored in the cooler overnight and allowed to warm to room temperature the next day before being weighed individually using a Mettler PL1200 electronic balance (Mettler Instrument Corporation, New Jersey, U.S.A.). Following this the eggs were broken open with albumen height (mm) being measured using an Ames-S-6428 micrometer dial gauge (B.C. Ames Company, Massachusetts, U.S.A.). All meat and blood spots noticed were recorded but due to the low incidence, no statistical analysis was done. One-half of each shell was washed and air dried before recording shell and membrane thickness. The dried shells were broken by hand into smaller pieces with measurements being taken on three pieces using an Ames-No.25E micrometer (B.C. Ames Company, Massachusetts, U.S.A.).

For all the eggs in each replicate measured over the two days, the egg weights and albumen heights were averaged out with those averages being used to calculate Haugh Units. Averages were also calculated for shell elasticity and shell and membrane thickness.

Total egg weights (per replicate) were recorded on three consecutive days (days 13-15) of each production period. The average egg weight was multiplied by the total number of eggs produced per period to calculate total egg mass per period per replicate.

D. Tibia Collection and Preparation Procedures

Tibia samples were taken at the start of the trial (period 1) from all ten birds on the initial balance trial (IBT) and at the end of the trial (period 9) from all 20 birds on the final balance trial (FBT). Birds were sacrificed by cervical dislocation after having been weighed live. The left tibia was removed and scraped clean of all adhering tissue and fibula. The clean tibias were then stored frozen until further analysis.

II. Balance Trial Procedures

Three balance trials each lasting seven days were conducted three times over the course of the experiment. The initial (pre-trial) balance trial (period 1, 26 weeks of age) involved ten birds individually caged and fed the standard pre-test layer diet one week prior to the start of the experiment. The midway (period 5, 46 weeks of age) balance trial and the final (period 9, 62 weeks of age) balance trial each involved five hens randomly selected from each treatment and individually caged. Each balance trial

was initiated at 14:00 hours after oviposition with birds having been fasted for 24 hours.

Each bird was supplied with 150 g of its respective diet on day one. Residual feed was weighed back every day at 14:00 hours and a new lot of 150 g was supplied.

Fecal collection trays covered with waxed paper were used to collect feces daily over 24 hour periods. The daily 24-hour fecal collections for individual birds were weighed, placed in polyethylene sample bags and stored frozen (-4^{o}C) until further analysis could be done.

All eggs laid during the balance trials were recorded and saved for analysis. Each egg was weighed and separated into white + yolk and shell + membranes. Contents were placed in polyethylene sample bags and stored frozen (-4^{o}C) for further analysis whereas the shell with membrane was air dried overnight, ground into a fine powder using mortar and pestle and stored in plastic vials for further analysis. Weights were recorded for separate components for each egg (Mettler PL1200 electronic balance, Mettler Instrumentation Corporation, New Jersey, U.S.A.).

Birds were weighed individually at the start and end of each balance trial. Upon completion of the IBT and FBT all birds were sacrificed by cervical dislocation, all feathers were removed and left tibias were excised and processed as previously described. The remaining carcass was frozen until further processing and analysis.

Before each balance trial a portion of each diet (enough to last through the trial) was thoroughly mixed to ensure uniformity within each diet. A sample was taken of each dietary treatment for further analysis.

III. Laboratory Procedures and Calculations

A. Introduction

All laboratory analysis was done by standard A.O.A.C. (1975) methods with the only modification being the use of wet-ashing technique (Thompson and Blanchflower, 1971) as opposed to dry-ashing for some materials in preparation for Ca and P analysis. This wet-ashing technique had the advantage of being quick, accurate and allowing for the testing of a large number of samples simultaneously. Samples were analyzed in duplicate with the results being averaged. In all cases practice samples were used previous to analysis on the actual experimental samples to ensure the procedure was accurate and consistent.

B. Sample Preparation

1. Feed. Each time feed was mixed a sample was taken of each of the four diets. For nutrient analysis an equal amount (by weight) of each diet sample was taken and mixed to provide one homogeneous sample for each of the four

diets. The feed was ground to a fine consistency through a stainless steel grinder (Wiley Models 2 and 3, Arthur H. Thomas Co., Philadelphia, U.S.A.) using a 2mm screen. Dry matter analysis was performed according to A.O.A.C. section 7.003.

2. Excreta. Frozen excreta samples were lyophilized for a period of 70-100 hours for dry matter determination. The two freeze-dryers used were a Virtis Freeze-Dryer Model 10-145LP-MR (The Virtis Company Inc., New York, U.S.A.) and a Labconco "Vac-Stop" Tray Dryer Model 75150 with a Labconco 18 Litre Freeze-Dryer Model 75015 (Labconco Corporation, Missouri, U.S.A.). These dried samples were then ground using a glass beaker and stainless steel spatula after which they were finely ground through a stainless steel grinder using a .7mm screen.

To compare P balance between days when hens laid an egg (ED) and days when hens did not lay an egg (NED) it was necessary to analyze the excreta samples separately. Excreta samples were composited for ED and NED for each hen on the balance trials. The samples were mixed thoroughly by hand in a glass beaker using a stainless steel spatula.

3. Eggs.

a. Egg Contents. The frozen egg contents of each egg laid in the balance period were lyophilized for a period of

24 hours for dry matter determination.

All lyophilized egg contents for each hen were then composited and mixed until homogeneous as described for the excreta samples. Due to the fine composition of the freeze-dried egg contents the samples were not ground but thoroughly mixed.

b. Egg Shells. All egg shell + membrane samples for each hen in the balance trials were then composited and thoroughly mixed before being further analyzed.

4. Carcasses. The frozen carcasses (minus feathers and left tibias) were cut into small cubes using a Hobart Meat Saw Model 5012 (Hobart Manufacturing Co. Ltd., Toronto, ON, Canada) and these cubes were fed through a Hobart Meat Grinder Model A200 (Hobart Manufacturing Co. Ltd., Toronto, ON, Canada) and mixed by hand. The grinding procedure was repeated four times following which a composite sample (approximately 170g) of each carcass was stored frozen in a polyethylene sample bag for further analysis.

Dry matter was determined by lyophilizing the samples for a period of 70-100 hours. After lyophilization each sample was again thoroughly mixed using a glass beaker and stainless steel spatula to ensure uniformity of the sample.

The fat content of each dry carcass sample was removed by ether extraction (Goldfish Apparatus) by the method of A.O.A.C. section 7.045 for a period of six hours giving dry,

fat-free carcass samples.

5. Tibias. The frozen tibias were autoclaved for 15 minutes, cooled and stripped of adhering tissue. They were then dried in a steam cabinet for 30 hours.

The dry tibias were cut in half with a stainless steel saw to expose the marrow and ensure more rapid and complete fat extraction. Fat was extracted with hexane by the A.O.A.C. method section 7.045 for 24 hours. The fat-extracted tibias were then dried in a steam cabinet for 24 hours to evaporate all the hexane giving dry, fat-free tibias for further analysis.

C. Chemical Analysis

1. Protein. Feed was analyzed for protein by the Kjeldahl method as outlined by A.O.A.C. sections 2.047-2.049. Each sample was tested twice in duplicate giving four values which were averaged out to give the final protein level.

2. Ash. The ash content of the composited feed samples was determined by A.O.A.C. method in section 7.010. This same procedure was used to determine the ash content of the composited egg shells, dry, fat-free carcass samples (550^{o}C for 6 hours) and dry, fat-free tibias.

3. Calcium. Feed Ca content was determined according to A.O.A.C. sections 7.077-7.085 using the wet-ashing technique modified by Thompson and Blanchflower (1971). This procedure was successfully tested out using an orchard leaf standard (Standard Reference Material 1571, National Bureau of Standards, U.S. Department of Commerce, Washington, D.C., Jan. 28, 1971) prior to being used for the feed samples.

4. Phosphorous

a. Wet-Ashing Technique. The P content of feed and eggshells was determined by the standard colorimetric method outlined in A.O.A.C. sections 2.019-2.025 following wet-ashing by the procedures of Thompson and Blanchflower (1971). This procedure was previously successfully tried out on orchard leaf standard and bovine liver standard (Standard Reference Material 1577a, National Bureau of Standards, U.S. Department of Commerce, Washington, D.C., June 15, 1982).

b. Dry-Ashing Technique. The P content of each of the composited ED and NED excreta samples, egg contents, carcasses and tibias was determined by the standard colorimetric method outlined in A.O.A.C. sections 2.019-2.025 following dry-ashing (for carcass and tibia dry-ashing see section II, C, 2). Dry-ashing was used on the excreta and egg contents after a single sample of each was tested seven times by each of a dry-ashing procedure and two wet-ashing procedures. The dry-ashing procedure provided the

most consistent results.

IV. Statistical Analysis

All non-balance trial data were subjected to two-way analysis of variance using the Statistical Analysis System (SAS, 1982) for computer processing of the data in order to determine the main and interaction effects of dietary P supplementation and protein source. Where treatments differed significantly they were compared by Tukey's test (SAS, 1982).

All balance trial data was tested for main effects and interactions using the general linear models procedure (SAS, 1982).

RESULTS

I. Performance of Laying Hens fed Diets Containing two Protein Sources with or without Supplemental P for 9 28-Day Periods

Overall averages of various production and performance parameters were analyzed by analysis of variance (ANOVA) to determine the effects of treatment, protein source, P supplementation level and protein by P interactions. To evaluate these effects for specific time periods some of the data were pooled in three groups of 84-day (3 x 28 days) periods for analysis.

A. Hen-Day Egg Production, Daily Feed Consumption and Feed Efficiency Data

Mean hen-day egg production data are reported in Table 9. The values are listed as % hen-day egg production determined by the following formula: # eggs laid ÷ #hen-days x 100. Mean feed consumption data are presented in Table 10 as g/hen/day. Table 11 shows mean feed efficiency data as g feed/g egg determined by the following formula: feed consumed (g) ÷ [# eggs laid x mean egg weight (g)].

Table 9. Mean Hen-Day Egg Production (%) of Hens Fed 2 Protein Sources With or Without Supplemental P over 9 28-Day Periods.

Diets #	Protein Source	Suppl. P (%)	Periods 1-3	4-6	7-9	1-9
Treatments			NS[1]	NS	NS	NS
1	SBM	0	92.4±3.31[2]	85.8±6.02	77.3±7.77	85.1±4.46
2	SBM	0.1	93.4±4.17	86.0±7.96	78.2±9.26	85.8±4.25
3	CM	0	93.5±2.28	86.0±5.35	74.4±8.73	84.5±4.95
4	CM	0.1	92.3±2.76	88.4±2.84	81.4±5.76	87.4±3.07
Protein Source			NS	NS	NS	NS
	SBM		92.9±3.70	85.9±6.87	77.7±8.33	85.4±4.25
	CM		92.9±2.55	87.2±4.35	77.9±8.04	85.9±4.26
Phosphorous Level			NS	NS	NS	NS
	0		93.0±2.83	85.9±5.54	75.9±8.18	84.8±4.60
	0.1		92.8±3.49	87.2±5.95	79.8±7.69	86.6±3.70
Protein X P Level			NS	NS	NS	NS

[1] NS = Not Significant (P>.10)
[2] Mean ± Standard Deviation.

Table 10. Mean Daily Feed Consumption (g/hen) of Hens Fed 2 Protein Sources With or Without Supplemental P over 9 28-Day Periods.

Diets			Periods			
#	Protein Source	Suppl. P (%)	1-3	4-6	7-9	1-9
Treatments			NS[1]	NS	NS	NS
1	SBM	0	111.9±3.83[2]	112.8±5.55	111.4±4.27	112.1±4.17
2	SBM	0.1	110.4±6.10	109.4±8.89	108.9±5.55	109.6±5.76
3	CM	0	110.2±3.88	110.0±4.02	108.8±7.76	109.6±4.70
4	CM	0.1	110.2±6.04	111.5±5.74	112.7±8.63	111.5±5.68
Protein Source			NS	NS	NS	NS
	SBM		111.2±5.02	111.1±7.41	110.1±4.95	109.9±5.05
	CM		110.2±4.94	110.8±4.88	110.7±8.24	110.6±5.17
Phosphorous Level			NS	NS	NS	NS
	0		110.0±3.86	111.4±4.92	110.1±6.25	110.9±4.50
	0.1		110.3±5.91	110.5±7.36	110.8±7.29	110.6±5.65
Protein X P Level			NS	NS	NS	NS

[1] NS = Not Significant (P>.10)
[2] Mean ± Standard Deviation.

Table 11. Mean Feed Efficiency (g Feed/g Egg) of Hens Fed 2 Protein Sources With or Without Supplemental P over 9 28-Day Periods.

Diets			Periods			
#	Protein Source	Suppl. P (%)	1-3	4-6	7-9	1-9
Treatments			NS[1]	NS	NS	NS
1	SBM	0	2.12±.061[2]	2.18±.151	2.29±.261	2.20±.107
2	SBM	0.1	2.09±.121	2.13±.095	2.27±.245	2.19±.152
3	CM	0	2.12±.061	2.16±.121	2.39±.249	2.24±.159
4	CM	0.1	2.11±.106	2.11±.080	2.22±.086	2.15±.074
Protein Source			NS	NS	NS	NS
	SBM		2.10±.095	2.15±.126	2.28±.247	2.20±.128
	CM		2.12±.084	2.13±.103	2.30±.203	2.20±.129
Phosphorous Level			NS	NS	NS	NS
	0		2.12±.059	2.17±.134	2.34±.254	2.22±.133
	0.1		2.10±.112	2.12±.086	2.24±.181	2.17±.119
Protein X P Level			NS	NS	NS	NS

[1] NS = Not Significant (P>.10)
[2] Mean ± Standard Deviation.

For these three production parameters there were no significant differences among treatments; between protein sources or between supplemental P levels as well as no significant protein by P interactions. This held true for all trimesters and the overall average in each case. The mean square (MS) values for hen-day egg production, daily feed consumption and feed efficiency are given in Appendix Table A1.

B. Egg Quality Measures

All cracked and shell-less eggs produced over the course of the trial were recorded and those numbers were converted to a percentage of the total egg production. The resultant data are reported in Table 12. There were no significant differences among treatments; between protein sources or between supplemental P levels as well as no significant protein by P interactions. In the final trimester (periods 7-9) there was a trend towards a protein by P interaction (P=.09). Phosphorous supplementation of the SBM diet resulted in a slight increase in the incidence of cracked and shell-less eggs while in the CM diet the P supplementation decreased the incidence of cracked and shell-less eggs considerably. The corresponding MS values are presented in Appendix Table A2.

Mean egg weight data are shown in Table 13. Over the first trimester (periods 1-3) there were trends towards

Table 12. Incidence of Cracked and Shell-less Eggs (%) of Hens Fed 2 Protein Sources With or Without Supplemental P over 9 28-Day Periods.

Diets			Periods			
#	Protein Source	Suppl. P (%)	1-3	4-6	7-9	1-9
Treatments			NS[1]	NS	NS	NS
1	SBM	0	.32±.249[2]	.72±.678	.84±.865	.59±.461
2	SBM	0.1	.35±.459	.40±.275	.87±1.059	.52±.341
3	CM	0	.32±.459	.63±.395	1.39±.674	.70±.250
4	CM	0.1	.53±.775	.46±.490	.54±.562	.51±.530
Protein Source			NS	NS	NS	NS
	SBM		.34±.359	.56±.530	.86±.941	.56±.396
	CM		.43±.569	.54±.442	.97±.746	.61±.414
Phosphorous Level			NS	NS	NS	NS
	0		.32±.240	.67±.542	1.12±.807	.64±.366
	0.1		.44±.627	.43±.388	.71±.842	.52±.434
Protein X P Level			NS	NS	X	NS

[1] NS = Not Significant (P>.10); X P<.10.
[2] Mean ± Standard Deviation.

Table 13. Mean Egg Weights (g) for Hens Fed 2 Protein Sources With or Without Supplemental P over 9 28-Day Periods.

Diets			Periods			
#	Protein Source	Suppl. P (%)	1-3	4-6	7-9	1-9
Treatments			X	NS	*	X
1	SBM	0	57.2±2.11	60.7±1.52	63.6±1.36a[1]	60.5±1.22
2	SBM	0.1	56.7±1.39	59.9±1.09	62.0±1.28a,b	59.9±1.31
3	CM	0	55.6±1.35	59.6±1.26	61.6±1.58b	58.9±0.99
4	CM	0.1	56.6±1.57	59.9±1.85	62.5±1.41a,b	59.7±1.50
Protein Source			X	NS	NS	X
	SBM		56.9±1.30	60.3±1.35	62.8±1.53	60.0±1.19
	CM		56.1±1.52	59.7±1.55	62.1±1.53	59.3±1.43
Phosphorous Level			NS[2]	NS	NS	NS
	0		56.4±1.50	60.1±1.48	62.6±1.74	59.7±1.47
	0.1		56.6±1.44	59.9±1.48	62.2±1.34	59.6±1.24
Protein X P Level			X	NS	**	*

[1] Mean ± Standard Deviation having different postscripts are significantly different.

[2] NS = Not Significant (P>.10); X P<.10; * P<.05; ** P<.01.

significant treatment differences (P=.09), significant protein source differences (P=.07) and a significant protein by P interaction (P=.08). Phosphorous supplementation of the SBM diet tended to decrease egg weights whereas in the CM diet it tended to increase egg weights. Through the second trimester (periods 4-6) there were no significant differences among treatments, between protein sources or between supplemental P levels as well as no significant protein by P interaction.

In the third trimester (periods 7-9) there was a significant treatment effect (P<.05) and a significant protein by P interaction (P<.01). Diet 1 yielded significantly larger eggs than diet 3. Phosphorous supplementation of the SBM diet decreased egg weights whereas in the CM diet it increased egg weights.

Over the entire trial (periods 1-9) there were trends towards significant treatment differences (P=.07) and significant protein differences (P=.09). There was a significant protein by phosphorous interaction (P<.05) where P supplementation of the SBM diet decreased egg weights whereas in CM diet it increased egg weights. Egg weight MS values are given in Appendix Table A2.

Eggshell elasticity, eggshell thickness and Haugh Unit data are reported in Table 14. For these three selected egg quality measures there were no significant differences among treatments, between protein sources or between supplemental

Table 14. Mean Values for Selected Egg Quality Measures of Hens Fed 2 Protein Sources With or Without Supplemental P over 9 28-Day Periods.

Diets			Quality Measures		
#	Protein Source	Suppl. P (%)	Eggshell Elasticity	Eggshell Thickness	Haugh Units
			um	mm	
Treatments			NS[1]	NS	NS
1	SBM	0	23.5±1.33[2]	.38±.01	84.5±2.26
2	SBM	0.1	24.0±1.78	.38±.01	84.9±1.40
3	CM	0	22.7±0.82	.38±.01	84.1±1.88
4	CM	0.1	23.9±2.00	.37±.01	84.8±2.20
Protein Source			NS	NS	NS
	SBM		23.8±1.55	.38±.01	84.7±1.84
	CM		23.8±1.49	.37±.01	84.5±2.02
Phosphorous Level			NS	NS	NS
	0		23.6±1.08	.38±.01	84.3±2.03
	0.1		24.0±1.84	.37±.01	84.8±1.79
Protein X P Level			NS	NS	NS

[1] NS = Not Significant (P>.10)
[2] Mean ± Standard Deviation.

P levels as well as no significant protein by P interaction. Corresponding MS values are presented in Appendix Table A2.

C. Body Weight Changes and Mortality Data

Live body weight and body weight change data are shown in Table 15. There were significant ($P<.05$) treatment and protein source differences along with a significant protein by P interaction ($P<.05$) for body weight gains between periods 1 and 5, while P supplementation level did not have a significant effect. Hens fed diet 1 gained more weight than those fed diet 3 and hens fed SBM diets gained more weight than those fed CM diets. Phosphorous supplementation of the SBM diet decreased body weight gains whereas it increased them in the CM diet.

There was a trend towards a significant protein by P interaction for body weight gains between periods 5 and 9 ($P=.06$). Phosphorous supplementation of the SBM diet tended to increase gains whereas it tended to decrease them in the CM diet. There were no significant treatment, protein source or P supplementation effects on gains between periods 5 and 9.

Body weight gains between periods 1 and 9 were significantly affected by protein source ($P<.05$) but not by treatment or supplementary P level. Hens fed SBM gained more weight than those fed CM. There was no significant protein by P interaction.

Table 15. Average Live Body Weights and Body Weight Gains of Hens Fed 2 Protein Sources With or Without Supplemental P over 9 28-Day Periods.

#	Protein Source	Suppl. P (%)	Initial Body Weight (Period 1)	Body Weight Gain (Period 1-5)	Midway Body Weight (Period 5)	Body Weight Gain (Period 5-9)	Final Body Weight (Period 9)	Body Weight Gain (Period 1-9)
			g					
Treatments			NS	*	NS	NS	NS	NS
1	SBM	0	1686±59[2]	174±54a	1860±80	16±37	1876±91	190±76
2	SBM	0.1	1669±76	130±107a,b	1799±122	40±75	1839±103	170±119
3	CM	0	1693±64	77±52b	1770±41	51±82	1821±113	128±79
4	CM	0.1	1660±61	127±64a,b	1787±71	-13±75	1774±81	114±80
Protein Source			NS	*	X	NS	X	*
	SBM		1678±66	151±85	1829±105	28±59	1858±97	180±98
	CM		1677±63	102±62	1779±58	18±83	1797±99	120±78
Phosphorous Level			NS	NS	NS	NS	NS	NS
	0		1690±60	125±71	1815±77	34±64	1849±104	159±82
	0.1		1665±67	128±86	1293±98	14±78	1807±96	142±103
Protein X P Level			NS	*	NS	X	NS	NS

[1] NS = Not Significant (P>.10); X P<.10; * P<.05.
[2] Mean ± Standard Deviation followed by different postscripts are significantly different.

Initial, midway and final live body weights were not significantly affected by treatment, protein source or supplemental P level and there was no significant protein by P interaction. The effect of protein source on body weight however did approach significance for the midway (P=.06) and final live body weights (P=.06). In both cases SBM diets yielded numerically heavier body weights than CM diets.

MS values for live body weights and live body weight gains are given in Appendix Table A3.

Mortalities were recorded as they occurred and are listed in Appendix Table A8 in chronological order along with diet, cage #, body weight and cause of death as determined by the Provincial Poultry Pathologist. Mortalities are presented in Table 16 by trimester and overall as birds/replicate. There were no significant differences among treatments, between protein sources or between P supplementation levels for any trimester or the overall mean. The protein by P interaction was not significant for any of the three trimesters but was significant (P<.05) for the overall mean. It did approach significance however for the second (periods 4-6) trimester (P=.09) and for the third (periods 7-9) trimester (P=.06). In each case the P supplementation of the SBM diet increased mortalities whereas it decreased mortalities in the CM diet. The corresponding MS values are shown in Appendix Table A3.

Table 16. Mortalities (Birds/Replicate) of Hens Fed 2 Protein Sources With or Without Supplemental P over 9 28-Day Periods.

Diets			Periods			
#	Protein Source	Suppl. P (%)	1-3	4-6	7-9	1-9
Treatments			NS[1]	NS	NS	NS
1	SBM	0	.10±.32[2]	.10±.32	.00±.00	.20±.42
2	SBM	0.1	.00±.00	.30±.48	.20±.42	.50±.53
3	CM	0	.00±.00	.20±.42	.30±.68	.50±.71
4	CM	0.1	.10±.32	.00±.00	.00±.00	.10±.32
Protein Source			NS	NS	NS	NS
	SBM		.05±.22	.20±.41	.10±.31	.35±.49
	CM		.05±.22	.10±.31	.15±.49	.30±.57
Phosphorous Level			NS	NS	NS	NS
	0		.05±.22	.15±.37	.15±.49.	35±.59
	0.1		.05±.22	.15±.37	.10±.31	.30±.47
Protein X P Level			NS	X	X	*

[1] NS = Not Significant (P>.10); X P<.10, * P<.05
[2] Mean ± Standard Deviation.

II. Short-Term (7-Day) P Balance of Hens fed 2 Protein Sources With or Without Supplemental P

A. Initial (Pre-Trial) Balance Trial

The IBT was conducted the last week before the long-term experiment started with ten hens fed the standard University of Manitoba laying hen diet. The purpose of the IBT was to determine some basic values for various parameters as well as become familiar with the procedures to be used in the midway and final balance trials. Since only a basal diet was employed, no statistical analysis was carried out.

1. Egg, Tibia and Carcass Measures. Data for selected egg, tibia and carcass measures are reported in Table 17.

2. P Balance Data. Phosphorous balance data is given in Table 18. The data show that early in their production cycle (producing at 98%) the hens were in a negative P balance losing 454 mg of P in seven days. The average daily losses of P were greater on egg days (80 mg) than on non-egg days (29 mg).

B. Midway (Period 5) Balance Trial (MBT)

1. Egg and Carcass Measures. Weights and % P for egg contents and eggshells are presented in Table 19. Treatment, protein source and P supplementation had no significant effects on %P of either egg content or eggshell.

Table 17. Mean Values for Selected Egg, Tibia and Carcass Measures of Hens fed the Standard University of Manitoba Laying Hen Diet over 7 Days During the Initial (Pre-Trial) Balance Trial (IBT).

Measure	Mean[1]
Egg (dry matter basis)	
Weight (g)	
Contents	11.34±1.17[2]
Shell	4.87±.42
Phosphorous (%)	
Contents	.71±.021
Shell	.20±.018
Tibia (dry, fat-free)	
Weight (g)	4.85±.274
Ash (%)	56.6±1.43
Ash P (%)	16.3±1.07
Carcass (dry, fat-free)	
Weight (g)	316.0±62.5
Ash (%)	20.4±2.32
Ash P (%)	14.1±1.04

[1] Each mean is derived from 10 observations.
[2] Mean ± Standard Deviation.

Table 18. Average Daily P Intake, Excretion, Egg Output and Retention on Egg Days, Non-Egg Days and Average 7-Day Total by Hens Fed the Standard University of Manitoba Laying Hen Diet over 7 Days During the Initial (Pre-Trial) Balance Trial (IBT).

Measure	Egg[1] Days Average	Non-Egg[2] Days Average	7-Day[3] Total
Intake (mg)	932±254[4]	667±241	6633±1539
Output			
Excreta (mg)	911±201	696±173	6454±1285
Egg (mg)	101±16	--	633±212
Total (mg)	1012±72	696±173	7087±1488
Retention (mg)	-80±72	-29±344	-454±392

1 Egg Days = days on which hens laid an egg.
2 Non-Egg Days = days on which hens did not lay an egg.
3 7-Day Total = addition of egg day and non-egg day values over one week for 10 replicates.
4 Mean ± Standard Deviation.

Table 19. Mean Values for Selected Egg and Carcass Measures of Hens Fed 2 Protein Sources With or Without Supplemental P over 7 Days During the Midway (Period 5) Balance Trial (MBT).

Rations			Egg (DM Basis)				Carcass (dry, Fat-Free)		
			Contents		Shell				
#	Protein Source	Suppl. P (%)	Weight	P	Weight	P	Weight	Ash	Ash P
			g	%	g	%	g	%	%
Treatments			--[1]	NS[3]	--	NS	--	NS	NS
1	SBM	0	13.0±1.03[2]	.76±.023	5.45±.53	.18±.014	320.8±26.3	15.5±2.31	16.7±.70
2	SBM	0.1	12.8±.69	.75±.029	5.70±.27	.19±.007	316.2±26.7	16.5±1.59	16.4±.52
3	CM	0	13.1±1.14	.74±.020	5.50±.62	.20±.015	320.3±41.4	15.9±.83	16.7±.65
4	CM	0.1	13.7±1.34	.75±.033	5.52±.68	.18±.017	344.6±32.6	15.2±1.37	16.4±.65
Protein Source			--	NS	--	NS	--	NS	NS
	SBM		12.9±.83	.75±.021	5.58±.42	.19±.011	318.5±25.1	16.0±1.95	16.6±.64
	CM		13.4±1.22	.75±.029	5.51±.61	.19±.018	332.5±37.4	15.6±1.13	16.5±.64
Phosphorous Level			--	NS	--	NS	--	NS	NS
	0		13.1±1.03	.75±.025	5.48±.54	.19±.015	320.6±32.7	15.7±1.65	16.7±.63
	0.1		13.3±1.12	.75±.026	5.61±.50	.18±.014	318.4±39.9	15.9±1.55	16.4±.56
Protein X P Level			--	NS	--	*	--	NS	NS

[1] Analysis of Variance not done on egg contents weight, egg shell weight, or carcass weight.
[2] Mean ± Standard Deviation.
[3] NS = Not Significant (P>.10); * P<.05.

There was not a significant protein by P interaction for egg contents P % but this interaction was significant for eggshell P % ($P<.05$). Phosphorous supplementation increased the eggshell P % in the SBM diet but decreased it in the CM diet. The MS values for these egg measures are shown in Appendix Table A4.

Dry, fat-free carcass weights, ash % and ash P % are given in Table 19 also. Within both of these measures there were no significant differences due to treatment, protein source or level of P supplementation as well as no significant protein by P interaction. The appropriate MS values are shown in Appendix Table A4.

2. P Balance Data. Phosphorous intake, output and retention was measured on days when hens laid an egg and on days when no eggs were laid. Addition of the egg day and non-egg day data over one week gave the pooled 7-day balance data. The data is reported in Table 20 with appropriate MS values being given in Appendix Table A5.

a. Egg Day P Balance Data. Egg day P intake was significantly affected by treatment ($P<.01$), protein source ($P<.05$) and level of P supplementation ($P<.001$) but there was no protein by P interaction. Hens fed diets 1 and 3 had lower P intakes than diet 4 fed hens. SBM fed hens relative to CM fed hens and unsupplemented diet fed hens relative to supplemented diet fed hens had lower daily P intakes.

Egg day P output via excreta was significantly affected

Table 20. Average P Intake, Excretion, Egg Output, and Retention on Egg Days, Non-Egg Days and Average 7-Day Total by Hens Fed 2 Protein Sources With or Without Supplemental P over 7 Days During the Midway (Period 5) Balance Trial (MBT).

Rations			Egg Days[1]					Non-Egg Days[2]			Pooled 7-Day Balance[3]		
	Protein	Suppl.		Output									
#	Source	P (%)	Intake	Excreta	Egg	Total	Retention	Intake	Output	Retention	Intake	Output	Retention
			mg/day								mg/week		
Treatments			**	*	--[6]	*	NS	**	*	NS	**	X	*
1	SBM	0	468±60a[4]	373±71a	124±12	497±82a	-29±27	433±49a	275±35a	158±51	3196±441a	3157±567	39±175a
2	SBM	0.1	638±72a,b	411±80a,b	119±24	530±102a,b	108±58	617±118a,b	407±104a,b	210±159	4441±541a,b	3516±498	925±335b
3	CM	0	543±83a	449±132a,b	122±20	571±144a,b	-28±96	453±103a	320±52a	133±70	3626±706a	3664±1006	-38±588a
4	CM	0.1	761±116b	608±105b	149±55	757±103a	4±87	735±167b	520±172b	215±118	5278±836b	4734±687	544±404a,b
Protein Source			*	**	--	*	NS	NS	NS	NS	*	*	NS
	SBM		553±109	392±74	122±18	514±89	39±84	515±126	333±98	181±102	3819±804	3336±538	483±531
	CM		652±149	529±141	135±42	664±171	-12±88	578±195	409±153	169±68	4452±1136	4199±989	253±566
Phosphorous Level			***	*	--	X	*	***	**	NS	***	*	***
	0		505±78	411±108	123±16	534±117	-29±67	443±76	297±48	146±59	3411±599	3410±815	1±411
	0.1		700±112	510±136	133±43	643±171	57±88	676±148	463±145	213±125	4860±797	4125±846	735±404
Protein X P Level			NS[5]	NS	--	NS	NS	NS	NS	NS	NS	NS	NS

[1] Egg Days = days on which hens laid an egg.
[2] Non-Egg Days = days on which hens did not lay an egg.
[3] Pooled 7-Day Balance = addition of egg day and non-egg day data over one week.
[4] Means ± Standard Deviation followed by different postscripts are significantly different.
[5] NS = Not Significant (P>.10); X P<.10; * P<.05; ** P<.01; *** P<.001.
[6] Egg Day egg P outputs were not statistically analyzed as differences were deemed due to egg size to a larger degree than to treatment.

by treatment ($P<.05$), protein source ($P<.01$) and P supplementation level ($P<.05$) but there was no significant protein by P interaction. Hens fed diet 1 excreted less P than those fed diet 4. Hens fed SBM relative to those fed CM and hens fed unsupplemented diets relative to those fed supplemented diets excreted less P.

Total P output on egg days was significantly affected by treatment and protein source but not by P level and there was no significant protein by P interaction. Again hens fed diet 1 had a lower P output than those fed diet 4 while the SBM fed hens put out less P than the CM fed hens. The effect of P level did approach significance ($P=.07$) in that the unsupplemented hens had a numerically lower P output than the supplemented hens.

Egg day P retention was not significantly affected by treatment, protein source or by a protein by P interaction.

There was a significant effect ($P<.05$) of P level, however, in that the hens fed unsupplemented diets retained less P than those fed supplemented diets.

b. Non-Egg Day P Balance Data. Non-egg day P intakes and outputs were significantly affected by treatment ($P<.01$ and $P<.05$ respectively) and by P supplementation ($P<.001$ and $P<.01$ respectively) but not by protein source. In both parameters the hens fed diets 1 and 3 had lower values than those fed diet 4 while the hens fed unsupplemented diets had lower values than those fed supplemented diets. In neither

case was there a significant protein by P interaction. Non-egg day P retention was not significantly affected by treatment, protein source or supplemented P level and there was no significant protein by P interaction.

c. Pooled 7-Day P Balance Data. Pooled weekly P intake was significantly affected by treatment ($P<.01$), protein source ($P<.05$) and P level ($P<.001$). Hens fed diets 1 and 3 had lower intakes than hens fed diet 4. Hens fed SBM relative to CM and those fed unsupplemented diets relative to supplemented diets had lower intakes of P.

The pooled weekly P output was significantly affected by protein source and P level ($P<.05$ in both cases) with the responses being similar to those seen for intake. The effect of treatment on P output approached significance ($P=.06$) with hens on diet 1 putting out numerically less P than those on diet 4.

Pooled weekly P retention was significantly affected by treatment and P supplementation level ($P<.05$ and $P<.001$ respectively) but not by protein source. Hens fed diets 1 and 3 retained less P than those fed diet 2 and the hens fed unsupplemented feed retained less P than those fed supplemented feed. There was no significant protein by P interaction for the pooled weekly P intake, output or retention.

C. Final (Period 9) Balance Trial (FBT)

1. Egg, Tibia and Carcass Measures. Weights and % P data for egg contents and eggshell are reported in Table 21. Percent P for egg contents and eggshell was not significantly affected by treatment or P supplementation level and there was no protein by P interaction. The effect of protein source on egg content P % approached significance (P=.08) with the SBM diets tending to yield a higher P content than the CM diets. The effect of protein source on eggshell P% was significant (P<.05) but here the CM diets yielded a higher P content than the SBM diets.

Dry, fat-free tibia weights, % ash data and % P in ash data are presented in Table 21. The tibia weights were significantly affected by treatment (P<.05), protein source (P<.05) and P supplementation level (P<.05) but there was no protein by P interaction. Hens fed diet 2 relative to diet 3, those fed SBM relative to CM and those fed supplemented feed relative to unsupplemented feed had larger tibias. Percent ash and % P in ash were not significantly affected by treatment, protein source or P level and there was no significant protein by P interaction.

Dry, fat-free carcass weight, % ash data and % P in ash data are presented in Table 21 also. Percent ash and % P in ash were not significantly affected by treatment protein source or P level and there were no significant protein by P interactions.

Table 21. Mean Values for Selected Egg, Tibia and Carcass Measures of Hens Fed 2 Protein Sources With or Without Supplemental P over 7 Days During the Final (Period 9) Balance Trial (FBT).

Rations			Egg (DM Basis)										
			Contents		Shell		Tibia (dry, Fat-Free)			Carcass (dry, Fat-Free)			
#	Protein Source	Suppl. P (%)	Weight	P	Weight	P	Weight	Ash	Ash P	Weight	Ash	Ash P	
			g	%	g	%	g	%	%	g	%	%	
Treatments			--[1]	NS[3]	--	NS	*	NS	NS	--	NS	NS	
1	SBM	0	14.0±.80[2]	.76±.007	6.02±.45	.18±.025	4.89±.314a,b[4]	59.8±1.23	17.0±.18	338.1±38.2	17.4±1.61	16.3±.92	
2	SBM	0.1	14.0±.92	.77±.025	5.62±.21	.18±.033	5.56±.414a	61.2±1.84	16.3±1.53	344.9±29.5	17.5±1.25	17.1±.17	
3	CM	0	12.8±1.12	.75±.014	5.24±.87	.22±.024	4.63±.630b	60.4±1.56	16.5±.87	309.1±18.3	17.0±1.22	16.5±.54	
4	CM	0.1	14.1±.87	.75±.019	6.06±.54	.20±.005	4.85±.446a,b	59.8±1.23	16.2±1.38	342.1±28.9	16.0±0.49	16.5±.67	
Protein Source			--	X	--	*	*	NS	NS	--	NS	NS	
	SBM		14.0±.78	.76±.017	5.87±.41	.18±.026	5.23±.475	60.5±1.64	16.6±1.10	341.5±32.3	17.5±1.36	16.7±.73	
	CM		13.5±1.17	.75±.016	5.65±.81	.21±.019	4.74±.528	60.1±1.36	16.3±1.10	325.6±28.7	16.5±1.01	16.5±.57	
Phosphorous Level			--	NS	--	NS	*	NS	NS	--	NS	NS	
	0		13.4±1.10	.75±.011	5.63±.77	.20±.029	4.76±.489	60.1±1.35	16.7±.66	323.5±32.0	17.2±1.37	16.4±.72	
	0.1		14.1±.82	.76±.023	5.90±.48	.19±.020	5.21±.551	60.5±1.66	16.2±1.38	343.5±27.6	16.8±1.18	16.8±.54	
Protein X P Level			--	NS	--	NS	NS	NS	NS	--	NS	NS	

[1] Analysis of Variance not done on egg contents weight, egg shell weight or carcass weight.
[2] Mean ± Standard Deviation followed by different postscripts are significantly different.
[3] NS = Not Significant (P>.10); X P<.10; * P<.05.

The appropriate MS values for these egg, tibia and carcass measures are shown in Appendix Table A6.

2. P Balance Data. Phosphorous intake, output and retention was measured on days when hens laid an egg and on days when no eggs were laid. Addition of the egg day and non-egg day data over one week gave the pooled 7-day balance data. The data are reported in Table 22 with corresponding MS values given in Appendix Table A7.

a. Egg Day P Balance Data. Egg day P intakes were significantly affected by treatment ($P<.001$), protein source ($P<.05$) and supplementary P level ($P<.001$). Hens fed diets 1 or 3 had a lower intake than those fed diets 2 or 4. Hens fed SBM diets relative to those fed CM and those fed unsupplemented diets relative to those fed supplemented diets had lower P intakes.

Egg day excreta P output was significantly affected by treatment and supplementary P level ($P<.001$ in both cases) but not by protein source. Hens fed diet 1 had a lower P output than those fed diets 2 or 4 and hens fed diet 3 had a lower P output than those fed diet 4. The hens on the unsupplemented diets had lower P outputs than those fed supplemented diets.

Egg day total P outputs were significantly affected by treatment ($P<.01$) and by supplemental P level ($P<.001$) but not by protein source. The responses seen were similar to those seen for egg day excreta P output.

Table 22. Average Daily P Intake, Excretion, Egg Output, and Retention on Egg Days, Non-Egg Days and Average 7-Day Total by Hens Fed 2 Protein Sources With or Without Supplemental P over 7 Days During the Final (Period 9) Balance Trial (FBT).

Rations			Egg Days[1]					Non-Egg Days[2]			Pooled 7-Day Balance[3]		
	Protein	Suppl.		Output									
#	Source	P (%)	Intake	Excreta	Egg	Total	Retention	Intake	Output	Retention	Intake	Output	Retention
			mg/day								mg/week		
Treatments			***	***	--		**	***	**	NS	***	***	**
1	SBM	0	474±70a[4,5]	384±52a	130±19	514±65a	-40±35a	447±66a	301±41a	146±74	3311±438a	3239±296a	72±222a
2	SBM	0.1	720±45b	582±24b,c	139±7	721±22b,c	-1±23a,b	570±108a	413±119a	157±97	4314±914b	3935±1082b	379±376a
3	CM	0	549±67a	441±60a,c	122±17	563±70a,c	-14±66a	392±221a	226±158a	126±76	3723±348a	3531±364a	192±273a
4	CM	0.1	832±102b	606±111b	123±17	729±128b	103±69b	811±131b	654±182b	157±201	5777±764b	4939±664b	838±328b
Protein Source			*	NS[6]	--	NS	*	NS	NS	NS	**	*	*
	SBM		566±140	458±111	134±16	592±119	-26±36	509±106	357±103	152±81	3813±858	3587±833	226±333
	CM		601±170	524±121	122±16	646±131	45±88	602±280	460±260	142±144	4750±1219	4235±898	515±444
Phosphorous Level			***	***	--	***	**	***	***	NS	***	**	**
	0		511±76	416±61	122±19	538±69	-27±52	419±156	283±110	136±72	3517±432	3385±349	132±243
	0.1		790±99	597±86	129±16	726±98	64±76	691±170	533±193	158±149	5046±1107	4437±998	609±441
Protein X P Level			NS	NS	--	NS	NS	*	*	NS	X	NS	NS

[1] Egg Days = days on which hens laid an egg.
[2] Non-Egg Days = days on which hens did not lay an egg.
[3] Pooled 7-Day Balance = addition of egg day and non-egg day data over one week.
[4] Means ± Standard Deviation followed by different postscripts are significantly different.
[5] NS = Not Significant (P>.10); X P<.10; * P<.05; ** P<.01; *** P<.001.
[6] Egg Day egg P outputs were not statistically analyzed as differences were deemed due to egg size to a larger degree than to treatment.

The retention of P on egg days was affected significantly by treatment ($P<.01$), protein source ($P<.01$) and supplementary P level ($P<.01$) and was negative except for the canola P supplemented diet (#4). Diets 1 and 3 yielded lower retentions than diet 4 with diet 2 being intermediate. Soybean diets relative to canola diets and unsupplemented diets relative to supplemented diets were also significantly lower.

For these four egg day balance parameters there were no significant protein by P interactions.

b. Non-Egg Day P Balance Data. Non-egg day P intakes and outputs were significantly affected by treatment ($P<.001$ and $P<.01$ respectively) and supplementary P level ($P<.001$ for both parameters) but not by protein source. For both parameters hens fed diets 1, 2 or 3 had lower P values than those fed diet 4 and hens fed unsupplemented diets had lower P values than those fed supplemented diets. There was a significant protein by P interaction for both parameters ($P<.05$). Phosphorous intakes and outputs increased when SBM diets were supplemented with P but increased even more when CM diets were supplemented with P.

Non-egg day P retentions were not significantly affected by treatment, protein source or supplemental P level and there was no significant protein by P interaction.

c. Pooled 7-Day P Balance Data. The pooled weekly P intakes, outputs and retentions were significantly affected

by treatment (P<.001, P<.001 and P<.01 respectively), protein source (P<.01, P<.05 and P<.05 respectively) and P level (P<.001, P<.01 and P<.01 respectively). Phosphorous intakes and outputs were higher for hens fed diets 2 or 4 over those fed diets 1 or 3. Phosphorous retentions were higher for hens fed diet 4 over those fed diets 1, 2 or 3. For all three parameters, the CM diets relative to the SBM diets and the unspplemented diets relative to the supplemented diets had higher P values. In no case was there a significant protein by P interaction but for the pooled weekly P intake the interaction did approach significance (P=.09). Supplementation of the CM diet resulted in greater increases in P intake than did supplementation of the SBM diet.

D. Summary of P Balance Trial Data

A comparison of the pooled 7-day P retention of hens in the three P balance trials is shown in Table 23. The only times that birds were in a negative P balance was pre-test for the IBT (-454 mg/week) and for diet 3 in the MBT (-38 mg/week).

A comparison of the total carcass P of hens in the three P balance trials is shown in Table 24. Although no statistical analysis was carried out, with a total body P level of approximately 650 mg/100 g it would seem that the birds were not in a state of P deficiency. Numerically the

Table 23. Pooled 7-Day P Retention of Hens in the 3 Experimental Balance Trials (IBT, MBT, FBT).

#	Diets: Protein Source	Diets: Suppl. P (%)	Diets: Dietary P (%)	P Retentions (mg): IBT (Period 1)	P Retentions (mg): MBT (Period 5)	P Retentions (mg): FBT (Period 9)
Standard Hen Layer[1]			.801	-454±392[2,3]	--	--
Treatments			--	*	**	**
1	SBM	0	.409	--	39±175a[4]	72±222a
2	SBM	0.1	.536	--	925±335b	379±376a
3	CM	0	.535	--	-38±588a	192±273a
4	CM	0.1	.623	--	544±404a,b	838±328b
Protein Source				--	NS[5]	*
	SBM		.473	--	483±531	226±333
	CM		.579	--	253±566	515±444
Phosphorous Level				--	***	**
	0		.472	--	1±411	132±243
	0.1		.580	--	735±404	609±411
Protein X P Level				--	NS	NS

[1] Standard University of Manitoba Laying Hen diet used for the IBT.
[2] The 10 hens in the IBT were producing at a rate of 98%.
[3] Means ± Standard Deviation followed by different postscripts are significantly different.
[4] NS = Not Significant (P>.10); * P<.05; ** P<.01; *** P<.001.

Table 24. Total Carcass P (mg P/100 g hen)[1] of Hens in the 3 Balance Trials (IBT, MBT, FBT).

#	Diets: Protein Source	Diets: Suppl. P (%)	Diets: Dietary P (%)	Total Carcass P (mg/100 g): IBT (Period 1)	Total Carcass P (mg/100 g): MBT (Period 5)	Total Carcass P (mg/100 g): FBT (Period 9)
Standard Hen Layer[2]			.801	720(10)[3]	--	--
Treatments						
1	SBM	0	.409	--	743(2)	655(5)
2	SBM	0.1	.536	--	606(2)	714(5)
3	CM	0	.535	--	672(3)	651(5)
4	CM	0.1	.623	--	591(3)	637(5)
Protein Source						
	SBM		.473	--	625(4)	685(10)
	CM		.579	--	632(6)	645(10)
Phosphorous Level						
	0		.472	--	653(5)	653(10)
	0.1		.580	--	599(5)	675(10)

1 Complete carcass weight minus the feathers.
2 Standard University of Manitoba laying hen diet used for the IBT.
3 Bracketed numbers are the number of observations on which the mean values are based.

hens in the FBT had a higher carcass P content than those in the MBT.

DISCUSSION

I. Performance of Laying Hens fed Diets Containing two Protein sources With or Without Supplemental P for 9 28-Day Periods

Hen-day egg production, daily feed consumption and feed conversion efficiency were unaffected by treatment within each trimester and overall with the average values reported for these parameters being 85.7%, 110.7 g/hen and 2.195 g feed/g egg respectively. These results suggest adequacy of wheat-barley-soy (.4% TP, .2% AP where AP = 100% of IP and 50% of plant P) and wheat-barley-canola (.53% TP, .26% AP) diets for supporting high levels of efficient egg production over a 9-month period without the need for supplemental IP. These low P requirements are in support of Owings et al. (1977) and Crowley et al. (1963) using corn-soy diets (.42 and .41% TP respectively) and Guenter (1980) using an unsupplemented wheat-oats-soy diet (.38% TP). They are however lower than many of the P levels cited by other authors such as Singsen et al. (1962) (.4% TP) and Walter and Aitken (1962) using an unsupplemented wheat-corn-oats-soy diet (.39% TP). The latter trial extended over a 44 week (28-72 weeks) period whereas the study now being

reported lasted for 36 weeks only (27-63 weeks of age). This may account for the different results as Walter and Aitken noted that no real differences between groups became evident until the latter half of the 44-week trial with production decreasing more rapidly in the group receiving the basal unsupplemented diet than in those groups fed supplemental IP. This tendency was noted in this trial also but was not significant (P>.10). Of greater significance is the fact that Walter and Aitken fed free choice oystershell along with 1.5% Ca in the diet allowing for variable Ca intake which could affect egg production and bone Ca resorption accompanied by endogenous P loss while in this trial the Ca content of the feed was kept constant at approximately 3.5%. The low P requirement indicated here relative to the higher P requirements determined by many authors (Table 1) underscores the fact that many variables are involved and these need to be addressed in each feeding situation to determine dietary P requirement.

Incidence of cracked and shell-less eggs, eggshell elasticity, eggshell thickness and Haugh Unit scores were also unaffected by treatment within each trimester and overall with the average values reported for these parameters being .58%, 23.78 um, .378 mm and 84.58 HU respectively. Again these results indicate the adequacy of wheat-barley-soy (.4% TP, .2% AP) and wheat-barley-canola (.53% TP, .26% AP) diets for maintaining reasonable egg

shell quality and egg interior quality without the need for supplemental IP. This is in support of findings by Hunt and Chancey (1970) (.38% TP), Salman et al. (1969) (.3% TP), Daghir and Farran (1983) (.15% AP) and many other researchers investigating egg shell quality (Tables 2, 3, 4). There was a trend (P=.09) however for the incidence of cracked and shell-less eggs to increase with time in the unsupplemented CM treatment relative to the supplemented CM treatment while this response to P supplementation was not seen in the SBM treatments. It is possible that with continuation of the trial the effect would have become significant suggesting that an unsupplemented wheat-barley-canola (.53% TP, .26% AP) diet cannot maintain adequate bone P reserves and requires P supplementation in the latter stages of the laying cycle for maintenance of shell quality. This is in contrast to the theory of phase feeding decreasing levels of P (Mikaelian and Sell, 1981; Waldroup and Hellwig, 1982; Guenter, 1980; Said and Sullivan, 1982.; Scheideler and Sell, 1986; Sell et al., 1987) and the differences could be explained by the following differences in criteria and experimentation: use of cracked and shell-less eggs as a criterion vs. egg production; use of wheat-barley-canola vs. corn-soy diets; analysis of data by trimester rather than just overall; relatively long length of the trial; relatively low levels of dietary AP; and a relatively high rate of egg production.

Egg weights were not affected significantly by P level or protein source although SBM constantly yielded larger eggs than CM (P=.07 in the first trimester; P=.09 overall). Since age of bird, rate of egg production, energy intake and protein intake were equal for all treatments it is possible that the amino acid profile of CM vs SBM caused the response. As a protein source SBM relative to CM contains higher levels of all of the essential amino acids except for cystine (Scott et al., 1982). Body size is also a determining factor in egg weight and since the SBM fed hens were heavier than the CM fed hens (see Table 15) this may be the factor responsible. In the third trimester hens fed the unsupplemented SBM diet yielded significantly ($P \leq .05$) heavier eggs than those fed the unsupplemented CM diet. This difference also approached significance in the first trimester (P=.08) and overall (P=.09). Addition of IP to both diets decreased this difference by decreasing egg size in the SBM diet while increasing egg size in the CM diet resulting in a significant ($P < .05$) protein by P level interaction for the third trimester and overall with this interaction approaching significance (P=.08) in the first trimester. This interaction remains unexplained.

The body weights of layers were unaffected by treatment through the trial. However, hens fed SBM tended to be heavier than those fed CM at the midpoint (P=.06) and end (P=.06) of the trial. Since dietary energy and protein

intakes were the same between treatments it is possible that amino acid balance (in a similar manner as that for egg weight) was the factor most likely responsible. It is also possible that energy content of CM is overestimated or that of SBM is underestimated, causing the decreased body weight. Similarly, the body weight gains (unaffected by IP level) were higher for hens fed SBM than those fed CM ($P<.05$ for periods 1-5 and 1-9), especially in the first half of the trial in the unsupplemented diets (diet 1 vs. diet 3). The significant ($P<.05$) protein by P level interaction for periods 1-5 which reversed over periods 5-9 ($P=.06$) can not be explained and requires further investigation.

Mortalities were unaffected by treatment in contrast to results reported by Garlich (1979) where .23% AP and .28% AP did not support liveability relative to .48% AP. That research however was in periods of heat stress (27-40^{o}C) and cannot be compared to this trial (18-20^{o}C). The tendency for IP supplementation to decrease liveability in the SBM diets but increase it in the CM diets ($P=.09$ in the second trimester; $P=.06$ in the third trimester; $P<.05$ overall) is puzzling and could simply be due to chance as a result of the small data base used.

II. Short-Term (7-Day) P Balances of Hens Fed 2 Protein Sources With or Without Supplemental P

A. Initial (Pre-Trial) Balance Trial (IBT)

Since the purpose of the IBT was to determine some basic values for various parameters as well as become familiar with the procedure to be used in the later balance trials only the pre-test barley-soy diet (.8% TP) was fed to ten hens and no statistical analysis was carried out.

Egg day (ED) P intakes were very high at 932 mg P/hen/day as were excreta outputs of 911 mg P/hen/day (see daily P requirements listed on Table 1). With a P output of 101 mg/egg the ED P output was 1012 mg/hen resulting in a net ED P loss of 80 mg/hen. The non-egg day (NED) intakes were considerably lower at 667 mg P/hen/day as were the excreta outputs of 696 mg P/hen/day resulting in a net loss of 29 mg P/hen/day. Since the birds were laying at a fairly high rate (86.3%) and so few (ten) were used for such a short time (seven days) there were very few NED from which to draw data, decreasing the accuracy of that data and making the comparison between ED and NED unreliable.

The data do indicate a greater loss of P on ED vs NED due to the P lost in the egg and increased endogenous P losses on ED due to shell calcification. If this egg P is factored in as retained P there would be a net P retention of 21 mg/hen/day on ED in keeping with findings by Mikaelian and Sell (1980) (22-69 mg/hen/day) and Scheideler and Sell (1986) (135-157 mg/hen/day) using similar dietary TP levels (.39-.69%).

Over the one week period of the IBT these hens were in a negative P balance of 454 mg TP/hen when including the egg TP (positive P balance of 179 mg TP/hen/week when excluding the egg TP) in support of findings by Mikaelian and Sell (1980). If this was extrapolated over 52 weeks of production the layers would be in a severe P deficiency (23,600 mg) and would lose more P than their body contains (Table 24). Under practical situations this does not occur with this barley-soy diet (.8% TP). As layers age their production drops as does their daily requirement for P (Sell et al., 1987) and their ability to utilize PP increases (Nelson, 1967). Since bone is in a constant flux and birds are continuously using body (bone) P reserves these layers will utilize bone P as required to maintain production especially over the short term (Garlich et al., 1975; Garlich, 1979). Also affecting P balance here could be the variety of barley (56-70% PP) and SBM (51-58% PP) used or storage conditions which affect PP content and phytase enzyme activity (Nelson, 1967) consequently affecting P availability and retention. Thus the extreme P deficiency noted in the IBT could be due to the high rate of egg production, the young age of the layers, the varieties of feedstuffs used and the small data base used.

B. Midway (Period 5) and Final (Period 9) Balance Trials (MBT) (FBT)

The data presented for the MBT and FBT is based on five birds per treatment group (ten birds per protein source and per supplemental P level) fed for seven days in each trial. Egg production was 78.1% in the MBT and 68.9% in the FBT leaving very few NED from which to draw data. This small data base and its effect on the significance or lack of significance of the results obtained must be kept in mind throughout the discussion.

1. Canola Meal vs. Soybean Meal. The layers sacrificed during the IBT (pre-trial) contained 448 mg P/tibia and 8537 mg P/carcass (calculated from Table 17). These values represent the starting P status' of the hens on this trial. After nine months in lay the birds in the FBT were sacrificed. Those fed CM contained 464 mg P/tibia and 9329 mg P/carcass while those fed SBM contained 525 mg P/tibia and 10,506 mg P/carcass (calculated from Table 21). Since body weight changes and tibia weight changes may have an effect on the carcass and tibia P contents they must be accounted for. This is done in Table 24 and these data show the SBM fed hens to be retaining and storing P (625 to 685 mg P/100 g body weight) while the CM fed hens in general maintained their P status (632 to 645 mg P/100 g body weight) (Table 24) over the course of the MBT and FBT. Both retention studies show very positive retentions and presumably bone storage of P for both protein sources (Tables 20 and 22).

Given similar feed intakes (110 vs 121 g/hen/day in the MBT; 117 vs 121 g/hen/day in the FBT) for CM and SBM but different TP contents (.58% in CM vs .45% in SBM) the CM fed hens consumed higher levels of TP. Fecal excretion and total output of P followed suit with the CM fed hens excreting higher P levels than those fed SBM. With the layers being adequate in bone P reserves (Table 24) the need for AP is limited to that required in the egg (approximately 128 mg) (Tables 20 and 22) and the endogenous P excretion. Retention of dietary P is thus to fill these two needs and once met would decrease with much of the excess dietary P being excreted. Dietary TP intakes in these balance trials were relatively high (Table 1) suggesting that the AP needs should be met quite easily with the excess P not being retained. Since layers fed the CM diets ingested more P above and beyond the requirement than those fed SBM they also excreted more P.

Since the overall weekly P retention was positive, as reported by Scheideler and Sell (1986) and Mikaelian and Sell (1980), for both protein sources for both balance trials, there must have been storage of P occurring. With bones being able to serve as reservoirs of P (Garlich et al., 1975) it would follow that the absorbed excess P (especially on NED) is deposited in the bones for future use resulting in higher tibia and carcass P contents as observed (Table 24).

Phosphorous retention was not significantly different between CM and SBM diets. This does not necessarily suggest that the P availabilities were equal between the two protein sources but rather could be a function of the positive P status of the birds and the high dietary P intakes. The data do indicate that the SBM fed layers were in a higher P balance than those fed CM (Table 24). This was reflected in the significantly larger tibias and numerically higher percentage for tibia ash, carcass ash and carcass ash P (Table 21) suggesting greater availability of SBM P over CM P for bone formation and maintenance.

The significantly ($P<.05$) higher retentions of P by hens fed CM over those fed SBM in the FBT (515 mg/hen/week vs 226 mg/hen/week) are opposite those seen in the MBT where SBM fed hens had higher ($P>.05$) P retentions than CM fed hens (483 mg/hen/week vs 253 mg/hen/week). Carcass data indicates a slight loss of body P reserves over the course of this nine month trial in those hens fed CM (208 mg) (632 to 645 mg P/100 g body weight in Table 24) and a substantial gain in those fed SBM (969 mg) (625 to 685 mg P/100 g body weight in Table 24) as calculated from Tables 17 and 21. The hens would be attempting to replenish these body P reserves if they were negative. Therefore, in the FBT one would expect the CM fed hens to be absorbing and retaining more P than the SBM fed hens in an effort to replenish lost body P reserves. This is observed in terms of actual P

retention (Table 22) but not in terms of % P retention (Table 25) probably due to the difference in absolute P levels between the two groups (.4 vs .53% unsupplemented) (.5 vs .63% supplemented).

2. Supplemented vs. Unsupplemented Diets. As noted in the previous section, the layers sacrificed during the IBT (pre-trial) contained 448 mg P/tibia and 9537 mg P/carcass (calculated from Table 17) with these values representing the starting P status' of the hens on this trial. After nine months in lay the birds fed supplemented diets (.565% TP) contained 511 mg P/tibia and 10,206 mg P/carcass while those fed unsupplemented diets (.465% TP) contained 478 mg P/tibia and 9603 mg P/carcass (calculated from Table 21). Based on the carcass data it appears that the supplemented hens had excess, highly available IP which was stored in the tibias in support of Garlich et al., 1975 and Garlich, 1979, increasing the body P reserves (669 mg) but not enhancing egg production, feed efficiency, egg size, egg quality or liveability significantly. The unsupplemented hens had adequate dietary P and increased body P reserves marginally (66 mg). Both the MBT and FBT retention studies (Tables 20 and 22) support these data.

The calculated P intakes were higher for the supplemented diets than the unsupplemented diets due to the higher daily feed intake (123 vs 105 g/hen in the MBT; 128 vs 108 g/hen in the FBT) coupled with the higher TP content

(.565% vs .465%). Phosphorous outputs were also higher for the supplemented diets over the unsupplemented diets for the same reasons as explained previously in the discussion of the CM vs SBM response.

Overall weekly P retentions were positive for both supplemental P levels in support of findings by Scheideler and Sell, 1986, for both balance trials suggesting that bone storage of P was occurring (see carcass data in Table 24) and that the unsupplemented diets were adequate in dietary TP. In both balance trials the retention of P was greater by the supplemented hens ($P<.001$ in MBT; $P<.01$ in the FBT) than by the unsupplemented hens. Since the difference in dietary P content was due solely to the addition of highly available IP it would appear that this P was absorbed and retained and deposited in the carcass resulting in higher retention values. Overall the P supplemented hens were in a positive P balance while the unsupplemented hens were in a marginally positive P balance (carcass data supported by balance data).

3. Egg Days vs Non-Egg Days. Phosphorous balance was measured separately for egg days (ED) and non-egg days (NED) in all three balance trials. Less P was retained on ED than on NED for all balance trials and this held true for both protein sources and both supplemental P levels. This difference in P retention was due to the P lost in the egg (average of 128 mg/egg) and the increased endogenous P

excretion from the egg formation (Choi et al., 1979) estimated at 70 mg/egg (Taylor and Kirkley, 1967). With this increased endogenous P loss one would expect less P retention on ED even after the egg P is factored in. This was observed in most cases here: CM = 12 vs 169 mg P in MBT; CM = 167 vs 142 P in FBT; SBM = 161 vs 181 mg P in MBT; SBM = 108 vs 152 mg P in FBT; supplemented diets = 190 vs 213 mg P in MBT and 193 vs 158 mg P in FBT; unsupplemented diets = 94 vs 146 mg P in MBT and 95 vs 136 mg P in FBT. The two cases where it did not happen (CM for FBT; supplemented diets for FBT) remain unexplained.

4. Estimations of P Availability from SBM and CM. The P availabilities of SBM and CM diets can be estimated by the following formula:

$$\left(\frac{\text{egg P + endogenous P + retained P}}{\text{P intake}}\right) \times 100;$$ where endogenous P = normal P loss (25 mg/day) + extra endogenous P loss due to egg production (70 mg/egg). The values arrived at in these balance trials are given in Table 25. In general the SBM diets yielded higher P availabilities than the CM diets except in the case of the FBT supplemented calculations. At that stage of production the hens fed SBM were yielding larger eggs than those fed CM consequently requiring more Ca for shell formation. If dietary Ca was limited then the extra needed Ca would have been reabsorbed from the bone, accompanied by an increased loss of endogenous P and a

Table 25. Apparent P Digestibilities (%) of SBM and CM Diets in the two Experimental Balance Trials (MBT, FBT).

	Protein Source	
	SBM	CM
	%	
MBT - unsupplemented	35.3	30.6
supplemented	49.5	34.9
FBT - unsupplemented	48.2	42.0
supplemented	23.3	33.4

resulting lower calculated retention of P. Thus in the latter stages of lay the importance of adequate dietary Ca is increased as it relates to P status and retention. Also at that stage, the CM fed hens were in a lower carcass P status relative to the start of the trial and would increase absorption of P to replenish lost body P reserves (Garlich et al., 1975).

It can be seen in Table 25 that absorption of P in the SBM and CM diets increased in the FBT relative to the MBT. Since older hens can better utilize PP than young hens (Nelson, 1967) the PP availability should be higher for these old layers than the hens used during the MBT as was observed.

The calculated P retention values noted in Table 25 (38%-65.4%) in general agree with those reported in previous literature (Das and Netke, 1979; Choi et al., 1979; Nwokolo et al., 1976; Nwokolo and Bragg, 1980; Guenter and Ward, 1986).

SUMMARY AND CONCLUSIONS

An experiment was conducted for 9 28-day periods to determine the performance of 320 layers (4 treatment groups x 80 layers/treatment group) fed wheat-barley-soy or wheat-barley-canola diets with (.5 and .63% TP respectively) or without (.4 and .53% TP respectively) supplemental IP.

Performance of layers (as measured by % hen-day egg production, feed intake, feed conversion efficiency, egg shell quality, egg interior quality, egg size and liveability) was not affected by dietary protein source or level of IP indicating equal productive value of CM and SBM and P adequacy in the unsupplemented experimental diets. There was a trend for SBM to increase body weight gains and egg weights over CM suggesting an improved amino acid availability and possibly an inflated book value for CM energy.

Three 7-day balance trials were conducted throughout the experiment [beginning (IBT), midpoint (MBT) and end (FBT)] to determine the P balance of birds fed the experimental diets or the standard University of Manitoba barley-soy laying diet. Data were pooled for days when hens

laid an egg (ED), days when hens did not lay an egg (NED) and combined.

Young hens were in a severe P deficiency (454 mg TP/hen/day) early in the pre-trial (IBT) possibly due to the high level of production (86.3%) and their inability to utilize PP in a barley-soy diet.

In the MBT and FBT the hens fed CM had higher intakes and outputs of P than those fed SBM due to the higher dietary TP level but effect of protein source on P retention was variable. In the MBT SBM yielded higher retention values while in the FBT CM yielded higher retention values. This could be due to lower production level later in the study, age of birds and their ability to utilize PP later in the study, carcass P status of the birds or experimental error. In both balance trials and for both protein sources P retentions were positive suggesting storage of P and this was supported by carcass data as P content increased over the course of the experiment for both CM and SBM. The fact that P retention was not significantly different between CM and SBM does not necessarily suggest that P availabilities were equal between the two protein sources but could be a function of the positive P status of the birds and high dietary TP intake. The data do indicate a higher P balance of SBM fed layers over the CM fed layers.

Again in the MBT and FBT hens fed supplemental IP had higher intakes and outputs of P than those fed the

unsupplemented diets again due to the higher dietary TP levels. The weekly P retentions were positive for both IP supplementation levels for both balance trials but were higher after P supplementation due to the addition of highly available IP. This was supported by carcass data as the unsupplemented layers maintained body P reserves while the supplemented layers increased body P reserves considerably. However the P supplementation did not enhance egg production, feed efficiency, egg size, egg quality or liveability.

Phosphorous retention was lower on ED than on NED due to the loss of P in the egg (101 mg/egg in IBT) (128 mg/egg in MBT and FBT) and increased endogenous P lost through shell calcification.

Egg, tibia and carcass measures were generally unaffected by treatment and hens were in a positive P balance during the MBT and FBT except for the hens fed the unsupplemented CM diet (.53% TP) which were in a state of marginal P deficiency in the MBT. These data indicate the adequacy of the unsupplemented wheat-barley-soy and wheat-barley-canola diets in terms of P for maintenance of body P status and hen production. The body P reserves were adequate and in general were higher for the FBT than the MBT.

The conclusions based on the results of this study are as follows:

(1) since supplementation of the wheat-barley-soy and wheat-barley-canola diets with .1% inorganic P increased body P reserves but had no effect on hen performance, this supplementation is not required for laying hen production;

(2) since the final body P reserves of hens on the unsupplemented diets were the same as the starting reserves, these unsupplemented diets are adequate in P for maintenance of performance and P status;

(3) since calculated apparent P digestibilities were higher in the FBT than in the MBT, older hens are better able to utilize plant P than young hens;

(4) since the calculated apparent P digestibilities were generally higher for SBM than CM and body P reserves increased more in the SBM fed layers than the CM fed layers, SBM is a better source of dietary P for laying hens than CM;

(5) since layers fed canola diets laid as well as those fed soy diets, CM is an adequate replacement for SBM in terms of egg production, feed efficiency, egg quality and liveability;

(6) since the SBM fed layers tended to produce heavier eggs and weigh more than the CM fed layers, SBM is a better protein source in terms of egg size and body weight.

Some more research needs to be done in the following areas in order to answer some further questions:

(1) replication of this trial over a full commercial production cycle (20 to 72 weeks of age) to ensure that length of trial is not a factor;

(2) replication of the balance trials with more birds (larger data base) used over a longer period (e.g. 2 weeks);

(3) investigation of the effect of protein source on egg size and layer body weight.

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APPENDIX TABLES

Table A1. Mean Squares of Hen-Day Egg Production (%), Daily Feed Consumption (g/Hen) and Feed Efficiency (g Feed/g Egg) of Hens Fed 2 Protein Sources With or Without Supplemental P over 9 28-Day Periods.

		Egg Production				Feed Consumption				Feed Efficiency			
		Periods				Periods				Periods			
Source	DF	1-3	4-6	7-9	1-9	1-3	4-6	7-9	1-9	1-3	4-6	7-9	1-9
Total	39	9.825	32.655	65.325	17.747	24.430	38.368	45.047	25.441	.008	.013	.050	.016
Treatments	3	4.170	15.408	82.664	15.115	7.012	22.225	37.738	15.859	.002	.009	.055	.013
Protein	1	.000	17.594	.378	2.704	9.608	1.037	3.630	.676	.001	.004	.005	.000
Phosphorous	1	.203	16.681	155.107	30.976	5.245	8.329	4.435	.676	.003	.022	.097	.025
Protein X P	1	12.308	11.948	92.507	11.864	6.183	57.308	105.151	46.225	.001	.000	.063	.015
Residual	36	10.296	34.092	63.880	17.966	35.882	39.713	45.656	26.240	.008	.013	.049	.016

Table A2. Mean Squares of Selected Egg Quality Measures of Hens Fed 2 Protein Sources With or Without Supplemental P over 9 28-Day Periods.

Source	DF	Incidence of Cracked & Shell-less Eggs (%) Periods 1-3	4-6	7-9	1-9	Mean Egg Weights (g) Periods 1-3	4-6	7-9	1-9	Mean Egg Quality Values Eggshell Thickness (mm)	Elasticity (um)	Haugh Units
Total	39	.223	.232	.796	.161	2.125	2.138	2.390	1.802	.146	2.251	3.649
Treatments	3	.104	.224	1.256	.072	4.486X	2.183	7.258*	4.134X	.105	.431	1.214
Protein	1	.081	.002	.125	.024	6.889X	3.306	4.624	4.830X	.169	.030	.506
Phosphorous	1	.141	.613	1.675	.155	.484	.380	1.225	.090	.144	1.190	2.862
Protein X P	1	.091	.058	1.968X	.036	6.084X	2.862	15.625**	7.482*	.001	.072	.272
Residual	36	.233	.233	.660	.168	1.928	2.134	1.993	1.607	.149	2.402	3.852

X P<.10; * P<.05; ** P<.01.

Table A3. Mean Squares of Average Live Body Weights (g), Body Weight Gains (g) and Mortalities (Birds/Replicate) of Hens Fed 2 Protein Sources With or Without Supplemental P over 9 28-Day Periods.

Source	DF	Initial Body Weights (Period 1)	Body Weight Gain (Period 1-5)	Midway Body Weights (Period 5)	Body Weight Gain (Period 5-9)	Final Body Weights (Period 9)	Body Weight Gain (Period 1-9)	Mortalities Periods 1-3	4-6	7-9	1-9
Total	39	.004	.006	.008	.005	.010	.009	.049	.131	.163	.276
Treatments	3	.002	.016*	.015	.008	.018	.013	.033	.167	.225	.425
Protein	1	.000	.025*	.026X	.001	.037X	.036*	.000	.100	.025	.025
Phosphorous	1	.006	.000	.005	.004	.018	.003	.000	.000	.025	.025
Protein X P	1	.001	.022*	.015	.019X	.000	.000	.100	.400X	.625X	1.225*
Residual	36	.004	.005	.007	.005	.010	.008	.050	.128	.158	.264

X P<.10; * P<.05.

Table A4. Mean Squares of Selected Egg and Carcass Measures of Hens Fed 2 Protein Sources With or Without Supplemental P over 7 Days During the Midway (Period 5) Balance Trial (MBT).

		Egg (DM Basis)		Carcass (Dry, Fat-Free)	
Source	DF	Contents P (%)	Shell P (%)	Ash (%)	Ash P (%)
Total	19	60.613	21.140	2.444	.372
Treatments	3	5.045	34.512	1.603	.221
Protein	1	6.125	.405	.808	.002
Phosphorous	1	4.805	12.005	.148	.659
Protein X P	1	4.205	91.125*	3.854	.002
Residual	16	71.033	18.633	2.602	.400

* P<.05.

Table A5. Mean Squares of Average Daily P Intake, Excretion, Egg Output and Retention on Egg Days, Non-Egg Days and 7-Day Total by Hens Fed 2 Protein Sources With or Without Supplemental P over 7 Days During the Midway (Period 5) Balance Trial (MBT).

Source	DF	Egg Days[1]				Non-Egg Days[2]			Pooled 7-Day Balance[3]		
		Intake	Output		Retention	Intake	Output	Retention	Intake	Output	Retention
			Excreta	Total							
		g									
Total	19	.019	.017	.024	.008	.026	.016	.024	1.023	.794	.279
Treatments	3	.080**	.053*	.067	.021	.090**	.050*	.011	4.224**	2.246X	.899*
Protein	1	.049*	.093**	.112*	.013	.018	.032	.008	2.006*	3.418*	.187
Phosphorous	1	.189***	.028*	.060X	.036	.241***	.112**	.000	10.439***	2.810*	2.441***
Protein X P	1	.003	.018	.029	.014	.001	.007	.024	.207	.511	.068
Residual	16	.007	.010	.015	.005	.013	.009	.026	.421	.522	.163

[1] Egg Days = days on which hens laid an egg.
[2] Non-Egg Days = days on which hens did not lay an egg.
[3] Pooled 7-Day Balance = addition of egg day and non-egg day data over one week.
X P<.10; * P<.05; ** P<.01; *** P<.001.

Table A6. Mean Squares of Selected Egg, Tibia and Carcass Measures of Hens Fed 2 Protein Sources With or Without Supplemental P over 7 Days During the Final (Period 9) Balance Trial (FBT).

Source	DF	Egg (DM Basis)		Tibia (dry, Fat-Free)			Carcass (dry, Fat-Free)	
		Contents P (%) 10^{-5}	Shell P (%) 10^{-5}	Weight (g)	Ash (%)	Ash P (%)	Ash (%)	Ash P (%)
Total	19	30.168	63.054	.310	2.206	1.169	1.596	.417
Treatments	3	48.008	120.855	.813	2.201	.672	2.268	.508
Protein	1	92.529X	301.629	1.181*	1.008	.432	4.456	.130
Phosphorous	1	26.522	31.174	1.013*	.764	1.260	1.058	.623
Protein X P	1	24.972	29.762	.246	4.831	.323	1.290	.772
Residual	16	26.345	50.668	.216	2.207	1.262	1.470	.400

X $P<.10$; * $P<.05$.

Table A7. Mean Squares of Average Daily P Intake, Excretion, Egg Output and Retention on Egg Days, Non-Egg Days and 7-Day Total by Hens Fed 2 Protein Sources With or Without Supplemental P over 7 Days During the Final (Period 9) Balance Trial (FBT).

		Egg Days[1]				Non-Egg Days[2]			Pooled 7-Day Balance[3]		
			Output								
Source	DF	Intake	Excreta	Total	Retention	Intake	Output	Retention	Intake	Output	Retention
		g									
Total	19	.027	.014	.016	.006	.029	.032	.020	1.283	.821	.168
Treatments	3	.128***	.054***	.054**	.020**	.129**	.132	.008	5.818***	2.756***	.565**
Protein	1	.036*	.008	.004	.016*	.103	.084	.007	4.394**	2.098*	.420*
Phosphorous	1	.345***	.151***	.156***	.037**	.239***	.250***	.011	11.680***	5.538**	1.133**
Protein X P	1	.001	.001	.002	.006	.047*	.062*	.007	1.379X	.633	.143
Residual	16	.006	.005	.007	.003	.009	.013	.023	.433	.458	.093

[1] Egg Days = days on which hens laid an egg.
[2] Non-Egg Days = days on which hens did not lay an egg.
[3] Pooled 7-Day Balance = addition of egg day and non-egg day data over one week.
X P<.10; * P<.05; ** P<.01; *** P<.001.

Table A8. Summary of Mortalities of Hens Fed 2 Protein Sources With or Without Supplemental P over 9 28-Day Periods.

Date	Diets #	Protein Source	Suppl P (%)	Cage	Body Weight	Cause of Death
07/04/83	1	SBM	0	51	1270	fatty liver syndrome
07/19/83	4	CM	0.1	57	1497	intestinal torsion
09/08/83	2	SBM	0.1	61	1211	peritonitis
10/14/83	2	SBM	0.1	76	1633	fatty liver syndrome
10/18/83	3	CM	0	53	2497	fatty liver syndrome
10/30/83	1	SBM	0	60	1361	fatty liver syndrome
11/08/83	3	CM	0	43	1950	fatty liver syndrome
11/15/83	2	SBM	0.1	59	1270	leukosis
11/28/83	3	CM	0	75	1361	pick-out
12/07/83	3	CM	0	49	1723	hypothermia
12/17/83	2	SBM	0.1	72	1361	leukosis
01/26/84	3	CM	0	75	1406	egg peritonitis
02/06/84	2	SBM	0.1	66	1497	egg peritonitis