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The Influence of an Exogenous Microbial Enzyme Supplement on Feed Consumption, Body Growth and Follicular Development of Pre-Lay Pullets on Maize-Soy Diets

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Abstract: An experiment was conducted to examine the benefits of supplementing maize-soy diets with a composite microbial enzyme, Allzyme SSF, for laying hens at the pullet stage. Seven hundred and twenty 12-week old Isa Brown pullets were used in the different level of protein content diets. Feed intake from 12 to 16 weeks of age was unaffected by the enzyme supplement, except at the lowest protein content ($P<0.05$). Feed intake was, however, affected by the enzyme supplement between 16 and 20 weeks ($P<0.001$) and over the entire trial period ($P<0.05$). Body weight at 16 weeks of age was reduced ($P<0.05$) on the control low-protein diet. Feed conversion ratio was not affected by dietary protein content or through supplementation with the microbial enzyme. The relative weight of the ovary was reduced ($P<0.01$) on the low protein control diet, but the weight of visceral organs associated with digestion was not affected. The pullets in all groups failed to come into lay at 17 weeks of age, mainly due to very severe winter, as the experiment was conducted in a house that was not completely temperature-controlled. Onset of lay occurred from about 23 weeks of age, after the hens were already on a commercial diet but effects of previous dietary treatments on early egg production were noticeable.

Key words: Exogenous microbial enzyme, egg production, layers and pullets

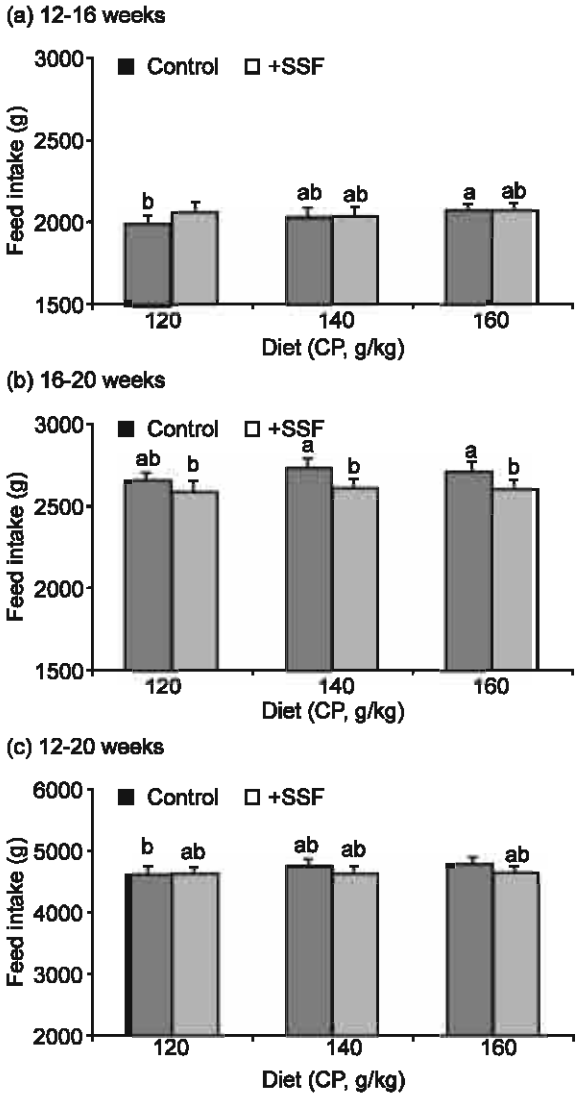
Introduction

Exogenous enzyme supplementation has been used commercially to improve nutrient digestibility of corn-soy diets. Bedford (1996) reported that exogenous enzyme supplementation resulted in an overall improvement in nutrient digestion and a reduction in endogenous amino acid losses. Marsman *et al.* (1997) also suggested that an improvement in the nutritional value of soybean meal could be achieved through supplementation with protease and non-starch polysaccharidase via improvement in digestibility of the non-starch polysaccharide fraction of soybean meal. Allzyme SSF is a commercially available feed enzyme, produced by a unique solid-state fermentation by non-genetically modified fungus - *Aspergillus niger*. It possesses the activities of seven enzymes (phytase, protease, pentosanase, β-glucanase, cellulase, amylase and pectinase), which are capable of breaking down protein, cellulose, pentosans, phytate and starch, therefore improving the digestibility and absorption of nutrients in the avian intestinal tract (Ramesh and Devegowda, 2004). Wu *et al.* (2003) suggested that preparations with multiple enzyme activities may provide a competitive strategy to improve nutrient utilization in poultry diets. The current study was conducted to examine whether feed intake, growth and reproductive development at the pullet stage is influenced by Allzyme SSF.

Materials and Methods

Ethical clearance: The Animal Ethics Committee of the University of New England (Armidale, NSW) approved this study.

Experimental birds and diets: Seven hundred and twenty (720) 12-week old Isa Brown pullets (initial weight, 848.7 ± 39.87 g) were obtained from a pullet grower based at Tamworth NSW, Australia. The study was conducted at the Laureldale Farm of the University of New England, Armidale NSW, in a modern open-sided layer house, enclosed with a blue poly-propylene blind. The birds were kept 4 per cage (40 cm x 50 cm), yielding a stocking density of 500 cm^2/bird. The birds were randomly allocated to three main pre-lay diets, which were iso-caloric (11.09 MJ ME/kg) but varied in crude protein content. The three diets contained 120, 140 or 160 g CP/kg (Table 1), each fed with or without a microbial enzyme supplement, Allzyme SSF (200 g/tonne), in a 3 x 2 factorial design. The three main diets are designated as Low-CP, Mid-CP and High-CP, respectively. Five contiguous cages constituted a replicate and there were 6 replicates per treatment. The study lasted a total of 8 weeks, during which the birds were kept on the pre-lay diets between 12 and 16 weeks of age and then transferred to a layer diet (11.09 MJ ME/kg and 160 g CP/kg). The birds were weighed at the start and thereafter every 2 weeks. Feed intake was also



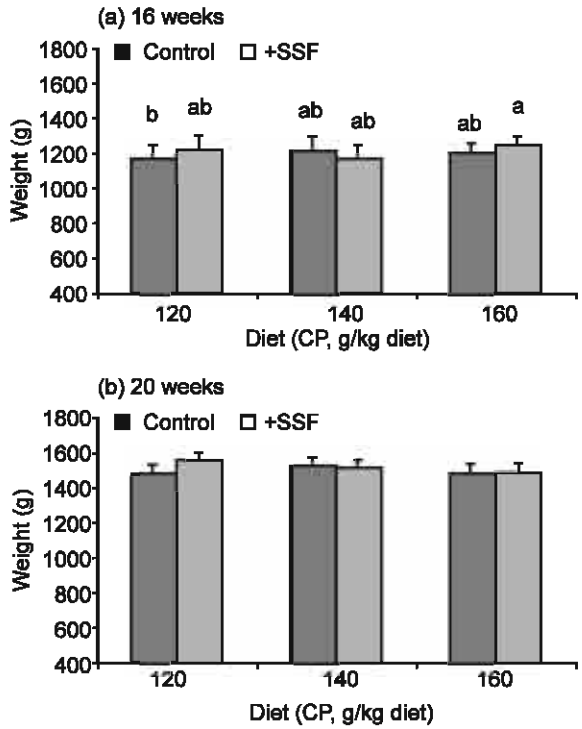
a,b-Mean values not sharing a superscript are significantly different (P<0.05).

Fig. 1: Feed intake of pullets on different and at different phases of feeding.

measured and feed conversion ratio (FCR) estimated from data on body weight gain and feed intake.

Sample collection: At 18 weeks of age, 2 birds per replicate were randomly selected and slaughtered through cervical dislocation. The birds were excised and the weight of various visceral organs (proventriculus /gizzard, small intestine, pancreas, reproductive tract, ovary, spleen and bursa) was recorded.

Statistical analysis: The data were analyzed by the General Linear Model procedure (MINITAB, 1998). Fisher's pair wise comparison and the t-test were used to compare the treatment means.



a,b-Mean values not sharing a superscript are significantly different (P<0.05).

Fig. 2: Body weight of pullets at 16 and 20 weeks of age.

Results

Feed intake: The feed intake for the different treatment groups at the different stages is as shown in Fig. 1. In the pre-laying phase (12-16 weeks of age), neither dietary protein nor enzyme supplementation separately had any significant effect on feed intake although the interaction between the two factors was significant (P<0.05). On the layer diet (16-20 weeks), supplementation with the microbial enzyme reduced (P<0.001) feed intake, particularly on the Mid- and High-CP diets. Over the entire trial period (12-20 weeks) the enzyme also tended to reduce feed intake but there were no significant differences between intakes at the same dietary level. Feed intake on the Low-CP control diet was significantly lower (P<0.05) than intake on the High-CP control diet.

Body weight: Body weight at 16 weeks of age was unaffected by dietary protein content or enzyme supplementation but there was an interaction (P<0.05) between the two factors (Fig. 2). On the same level of crude protein, enzyme supplementation only had marginal effects, tending to increase weight except on the mid-CP diet. Body weight on the Low-CP control diet was significantly lower (P<0.05) than on the High-CP enzyme-supplemented diet. There was no significant effect of crude protein, enzyme supplementation or their interaction on body weight at 20 weeks of age.

Table 1: Composition of experiment diets (g/kg)

	Pre-lay diets			
	Low-CP	Med-CP	High-CP	Layer diet
Corn	515.43	488.85	462.26	589.63
Wheat Flour	90.00	90.00	90.00	-
Millrun 14%	249.76	222.84	195.91	80.53
Soybean meal 48%	70.60	124.54	178.47	209.91
Tallow	5.00	5.00	5.00	5.00
Limestone 38%	30.78	30.60	30.42	73.37
Celite	20.00	20.00	20.00	20.00
Dicalcium phosphte	11.03	11.05	11.06	14.46
Sodium bicarbonate	1.11	1.00	0.88	0.72
DL-Methionine	0.33	0.47	0.61	1.36
Salt	2.42	2.49	2.56	2.87
Choline chloride 60%	1.17	0.92	0.67	0.15
L-Threonine	0.36	0.26	0.16	-
Premix	2.00	2.00	2.00	2.00
Total Batch (kg)	1000.00	1000.00	1000.00	1000.00
Nutrients (g/kg)				
AME (MJ/kg)	11.09	11.09	11.09	11.09
Protein	120.0	140.0	160.00	160.00
Calcium	15.0	15.0	15.00	32.00
Total phosphorus	6.6	6.6	6.60	6.20
Avail. Phosphorus	3.3	3.3	3.30	3.50
Lysine	5.1	6.5	7.90	8.10
Met + Cys	4.6	5.3	6.00	6.80
Threonine	4.5	5.2	6.00	6.10
Tryptophan	1.4	1.7	2.00	1.90

Note: Each diet was fed with or without Allzyme SSF (200g / ton feed).

Feed conversion ratio: Between 12 and 16 weeks of age, feed conversion ratio, in terms of feed intake per unit body weight gain was marginally reduced on the Low- and High-CP diets but not on the Mid-CP diet, as a result of enzyme supplementation. Over the early period on the layer diet (16-20 weeks of age) and over the entire trial period, the enzyme supplement tended to reduce FCR, but this effect was not significant (Fig. 3).

Visceral organ weight: The relative weight of visceral organs associated with digestion and immunity, at 18 weeks of age, is as shown in Fig. 4 and 5. Visceral organ weight was not affected by dietary protein or enzyme supplementation at any of the periods evaluated. On the Low- and Mid-CP diets, the weight of the proventriculus/gizzard was marginally reduced through enzyme supplementation but the reverse was the case for the weight of the small intestine. The relative weight of the pancreas was similarly increased on the Low- and High-CP diets while the weight of the spleen was slightly increased on all diets as a result of supplementation with the microbial enzyme.

Ovary weight and egg production: The relative weight of the ovary at 18 weeks of age was increased ($P<0.05$) by supplementation with microbial enzyme at the low and high CP contents (Fig. 6). Follicular development did not seem to be affected by treatment but follicular maturation appeared to be more rapid in pullets (not obvious at high CP) on the enzyme-supplemented diets (Plate 1). There was no significant effect of dietary protein content except

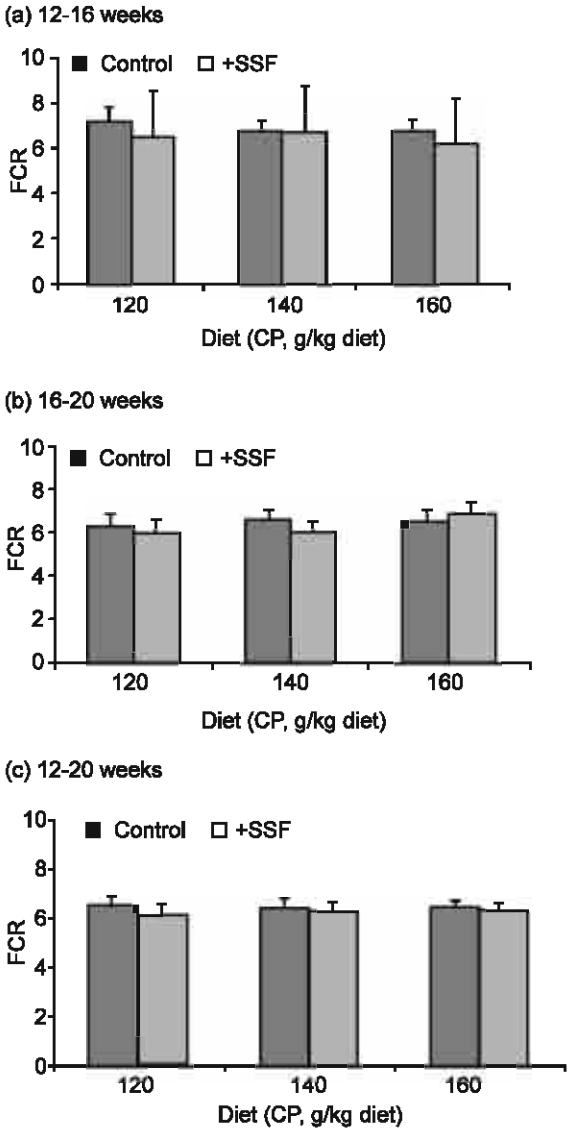


Fig. 3: Feed conversion ratio (FCR) of pullets on different diets at different phases of feeding.

between the Low-CP control and High-CP enzyme-supplemented diets, with pullets on the latter having significantly heavier ($P<0.05$) ovaries than the former. At high CP, the microbial enzyme supplement also tended to increase ovary weight but this was not significant. The supplement had a marginally negative effect in pullets on the Mid-CP diet.

Some of the pullets on the diet with SSF supplementation began to lay before the groups without SSF. This trend appeared to continue and records of egg production kept after the end of the study, when the hens had been moved on to a commercial diet, showed that egg production was much improved on the SSF-supplemented diets, but more so on the Low-CP diet

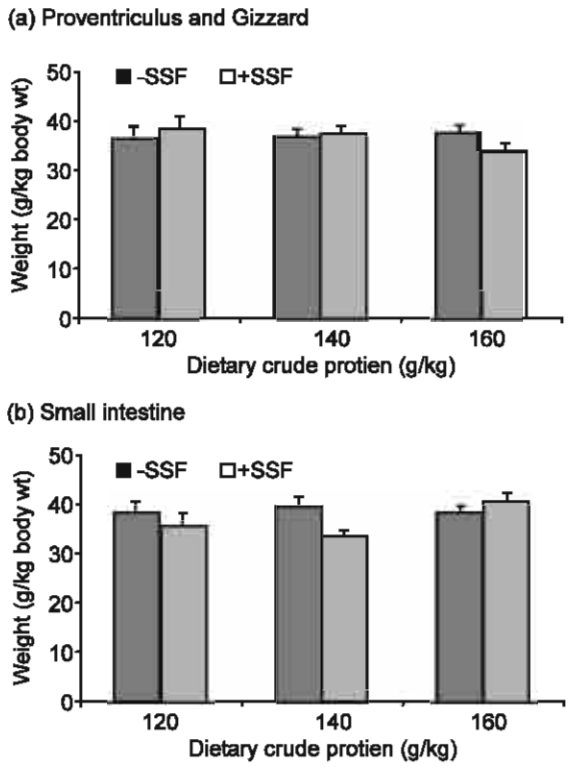


Fig. 4: Relative weight of proventriculus/gizzard and small intestine (g/kg body weight) at 16 weeks of age.

(Fig. 7). At 23 weeks of age, hen-day egg production was about 13.5, 40.5, 6.3, 22.2, 7.2 and 32.5 %, respectively on the Low-CP control, Low-CP enzyme-supplemented, Mid-CP control, Mid-CP enzyme-supplemented, High-CP control and High-CP enzyme-supplemented diets.

Discussion

The current results demonstrated that feed intake, body weight and FCR at any level of dietary protein are not significantly affected by Allzyme SSF. However, between 12 and 16 weeks of age, feed conversion ratio, was marginally improved on the Low- and High-CP diets as a result of enzyme supplementation. Over the early period on the layer diet (16-20 weeks of age) and over the entire trial period, the enzyme supplement tended to reduce feed conversion ratio, probably as a result of the fact that the birds were still growing. The improved FCR may be due to the reduction of intestinal viscosity and nutrient digestibility that has been improved by enzyme addition. Sundu *et al.* (2006) also suggested that the inclusion of enzyme in broiler diet based on copra meal can significantly increase feed conversion efficiency, DM digestibility, nutrient digestibility and decrease jejunal content viscosity.

Visceral organs and enzyme activity were not significantly affected by the inclusion of Allzyme SSF at

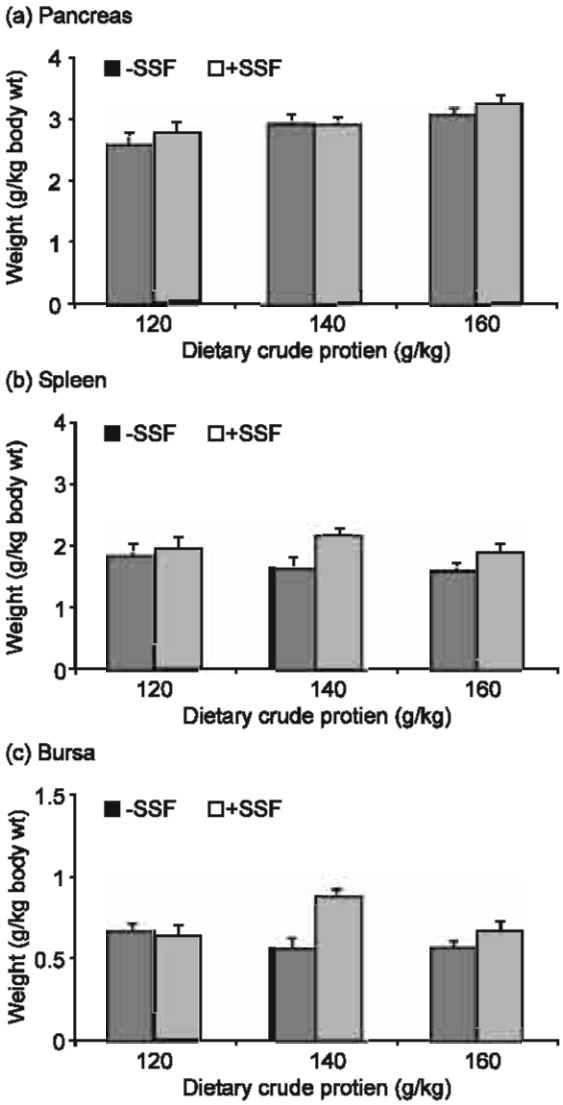


Fig. 5: Relative weight of pancreas, spleen and bursa (g/kg body weight) at 16 weeks of age.

any level of protein in the diet. Similar to these observations, Iji *et al.* (2003) also reported that the weight of visceral organs, protein content and activities of pancreatic and jejunal digestive enzymes were unaffected by microbial enzyme supplementation.

The data from the current trial showed that the protein level in the diet affected follicular development and egg production. Low-protein diets can result in a reduction in egg production. Soares *et al.* (2003) suggested that high quality protein with adequate amino acid balance is one of the most important factors for egg production in poultry. In the present study, the addition of Allzyme SSF to the low protein diet improved egg production due to the improvement in feed conversion ratio. It is also most likely that the digestibility of the diets was improved through enzyme supplementation, although this could

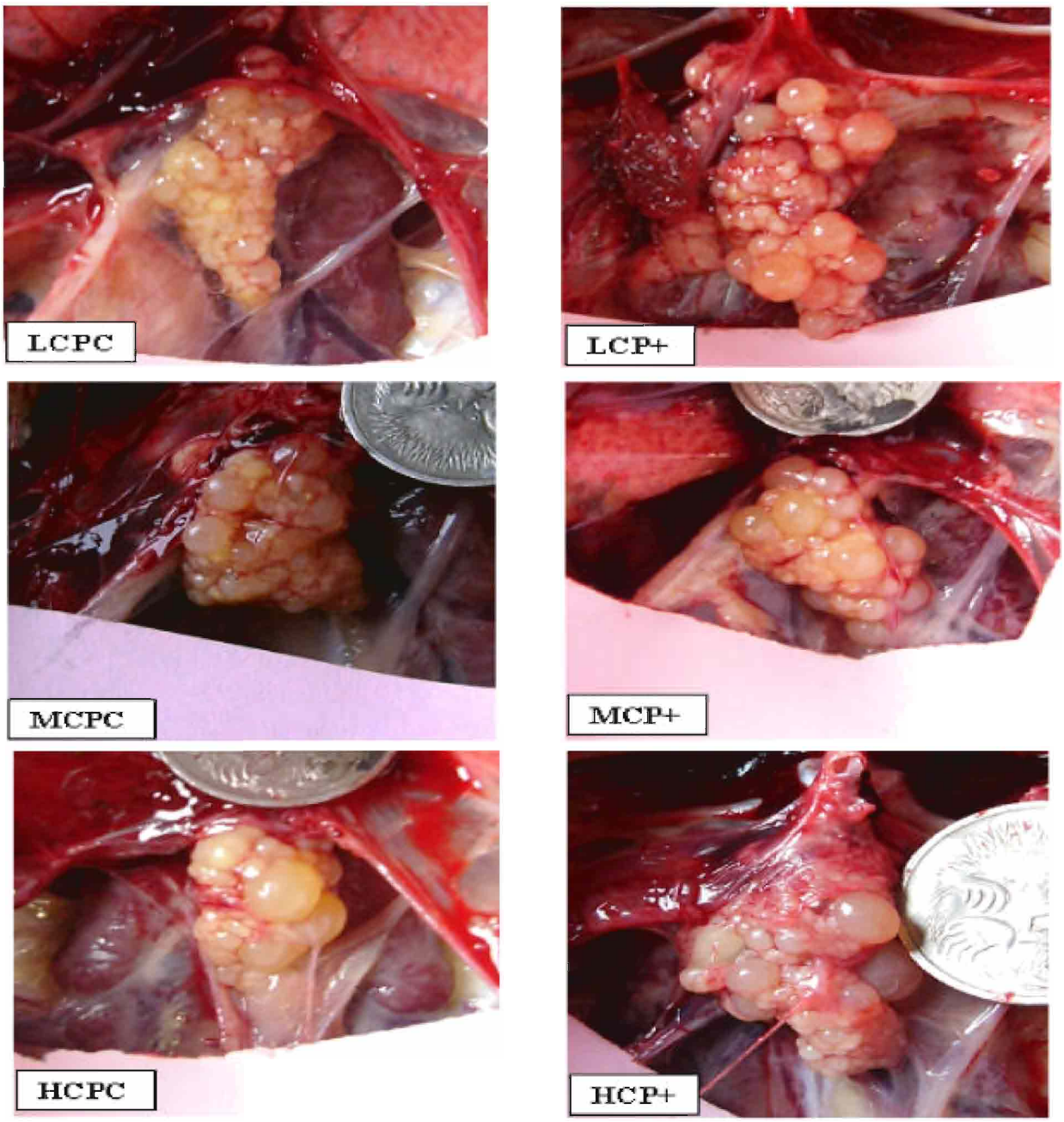


Plate 1: Follicular development in hens on different diets at 20 weeks of age. LCPC is low CP control, LCP+ is low CP + SSF, MCPC is mid-CP control, MCP+ is mid-CP + SSF, HCPC is high-CP control and HCP+ is high-CP + SSF.

not be measured due to problems with marker analysis. Ishibashi and Yonemochi (2003) also suggested that the availability of amino acid may be improved by feed management, especially supplementation with microbial enzyme.

Conclusion: The current results demonstrated that feed intake, body weight and FCR at any level of dietary protein are not significantly affected by Allzyme SSF. However, between 12 and 16 weeks of age, FCR, was marginally improved on the Low- and High-CP diets as a result of enzyme supplementation. The better FCR may be due to and improvement in nutrient digestibility in the presence of the enzyme supplement. Visceral

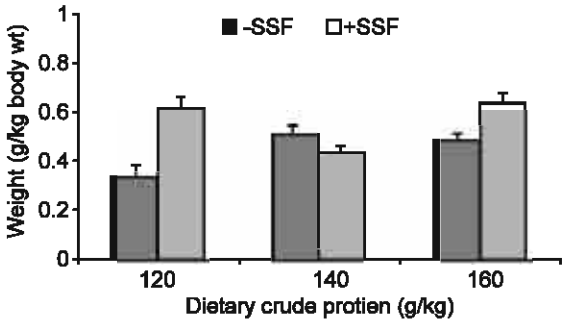


Fig. 6: Ovary weight (g/kg body weight) at 16 weeks of age

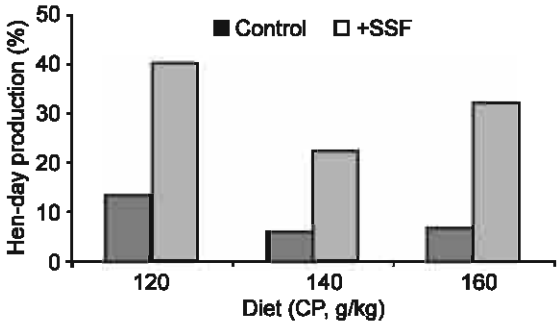


Fig. 7: Hen-day egg production at 23 weeks of age. Note that the hens had been moved on to a commercial layer diet at 20 weeks of age

organs were not significantly affected by the inclusion of Allzyme SSF at any level of protein in the diet.

From the results obtained in this study, the negative effect of low dietary protein content on follicular development was evident. However, supplementation of Allzyme SSF was found to improve the weight of follicle. Supplementation with the microbial enzyme at the pullet stage was also shown to enhance subsequent egg production.

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