

## Change in Body Weight and Ovary Weight of Pre-lay Pullets Fed Maize-soy Based Diet Supplemented with an Exogenous Microbial Enzyme

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### ABSTRACT

The experiment was conducted to examine the benefits of supplementing maize-soy diets with a composite microbial enzyme, Allzyme SSF<sup>®</sup>, for laying hens at the pullet stage. Seven hundred and twenty (720) 12-week old Isa Brown pullets were treated in the different level of protein content diets. The three diets contained 120, 140 and 160 g CP/kg, each fed with or without a microbial enzyme supplement, Allzyme SSF<sup>®</sup> (200 g/ton), in a 3 x 2 factorial design. The three main diets were designated as Low-CP, Mid-CP and High-CP, respectively. In experiment, 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. 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. The current results suggest that supplemental Allzyme SSF is beneficial to growing pullets on low protein diets, and the supplement supports egg production in early lay.

**Key words:** egg production, exogenous microbial enzyme, layers

### INTRODUCTION

Exogenous enzyme supplementation has been used commercially to improve nutrient digestibility of corn-soy diets. Bedford (1996) reported that the 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 the improvement in the nutritional value of soybean meal could be achieved by using protease and non-starch polysaccharide (NSP) enzyme supplementation via improvement in digestibility of the non-starch polysaccharide fraction of soybean meal. Allzyme SSF<sup>®</sup> is a commercially available feed enzyme, produced by a unique solid-state fermentation by non-genetically modified fungus - *Aspergillus*

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*niger*. It possesses the activities of seven enzymes (protease, pentosanase,  $\beta$ -glucanase, cellulase, phytase, amylase and pectinase), which are capable of breaking down protein, pentosans, cellulose, phytate and starch, therefore improving the digestibility and absorption of nutrients in the avian intestinal tract (Ramesh and Devegowda, 2004). Wu *et al.* (2003) reported that addition of Allzyme SSF (500 PU/Kg diet) to low phosphorus diets (0.3%) improved weight gain and feed intake, and reduced feed:gain ratio in broiler chickens. *In vitro* studies have revealed that enzymes produced by solid-state fermentation (SSF) are more efficient in releasing the nutrients than enzymes produced by regular submerged liquid fermentation (Robinson *et al.*, 2001). Allzyme SSF enzyme released more phytate-bound phosphorus (11.0% and 7.8% in wheat and maize-based diets, respectively) and  $\alpha$ -amino nitrogen (1.7% and 6.2% in wheat and maize-based diets, respectively) than non-SSF enzymes (Wu *et al.*, 2003). Wu *et al.* (2003) suggested that preparations with multiple enzyme activities may provide a competitive strategy to improve nutrient utilization in poultry diets. The aim of this study was conducted to examine whether feed intake, growth or reproductive development at the pullet stage was influenced by Allzyme SSF.

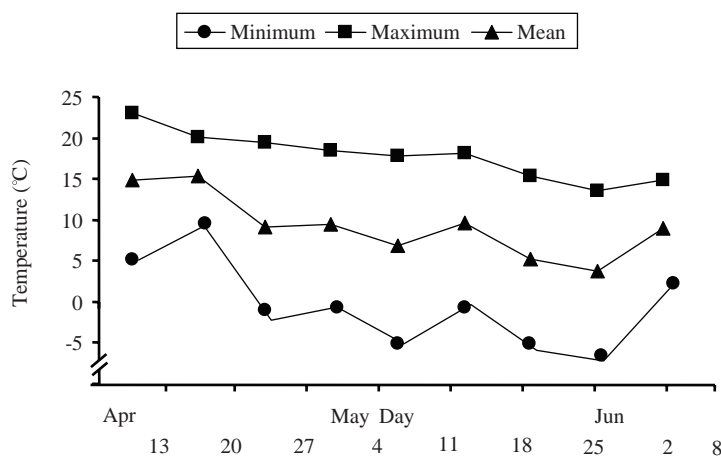
## MATERIALS AND METHODS

### Ethical clearance

This study has been approved by The Animal Ethics Committee of the University of New England (Armidale, New South Walls).

### Experimental birds and diets

Seven hundred and twenty (720) 12-week old Isa Brown pullets (initial weight,  $848.7 \pm 39.87$  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. Environmental conditions were not fully regulated, and the general temperature conditions of the area over the experimental period were shown in Figure 1. The birds were kept 4 per cage (40 cm  $\times$  50 cm), yielding a stocking density of 500 cm<sup>2</sup>/bird. In one replicate contain five contiguous cages and there were 6 replicates per treatment. Hence, in one treatment contained 120 birds in total. 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 (CP) (Table 1). The three diets contained 120, 140 and



**Figure 1** Temperature conditions in Armidale over the study period (13 April to 8 June 2006).

160 g CP/kg, each fed with or without a microbial enzyme supplement, Allzyme SSF® (protease, xylanase, cellulase, phytase, amylase,  $\beta$ , glucanase and pectinase) (200 g/ton), in a  $3 \times 2$  factorial design (6 treatments). The three main diets were Low-CP, Mid-CP and High-CP, respectively.

The study lasted a total of 8 weeks, during 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 until the end of experiment. Feed intake was also measured and feed conversion ratio (FCR) estimated from data.

### Sample collection

At 18 weeks of age, 2 birds per replicate were randomly selected and slaughtered by cervical dislocation. The birds were excised and the weight of ovary was recorded. After 20 weeks of age hens had been moved on to a commercial diet for 3 weeks. Egg production was record until 23 weeks of age.

### Statistical analysis

The data were analysed by the General Linear Model procedure (MINITAB, 1998). Fisher's pairwise comparison and the t-test were used to compare the treatment means.

**Table 1** Composition of experimental 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 phosphate	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	1000	1000	1000
<i>Nutrients (g/kg)</i>				
AME (MJ/kg)	11.09	11.09	11.09	11.09
Protein	120.0	140.0	160.0	160.0
Calcium	15.0	15.0	15.0	32.0
Total phosphorus	6.6	6.6	6.6	6.2
Avail. Phosphorus	3.3	3.3	3.3	3.5
Lysine	5.1	6.5	7.9	8.1
Met + Cys	4.6	5.3	6.0	6.8
Threonine	4.5	5.2	6.0	6.1
Tryptophan	1.4	1.7	2.0	1.9

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

## RESULTS

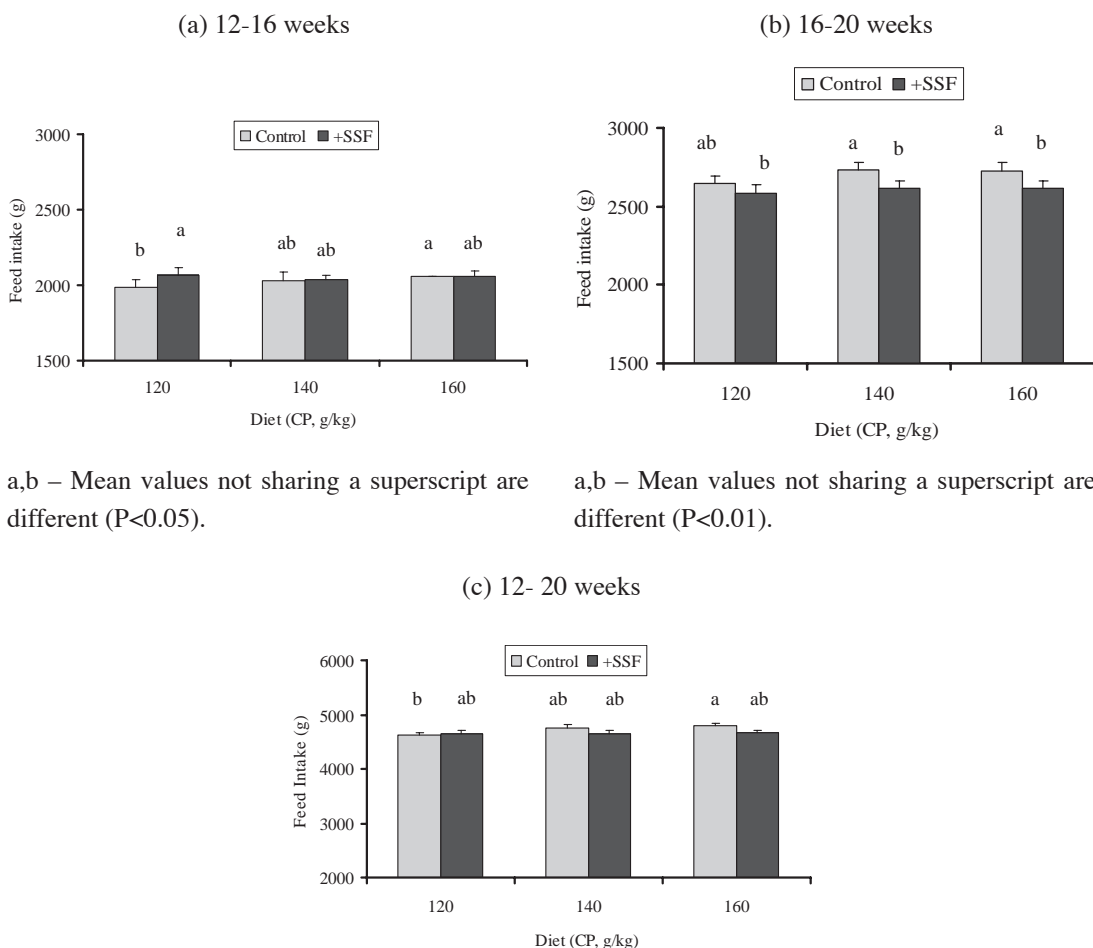
### Feed intake

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 significantly ( $P<0.05$ ). 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 diet groups (Figure 2). Over the entire trial period (12-20 weeks) the enzyme

tended to decrease feed intake but there were no significant differences between intakes at the same dietary level. Feed intake on the Low-CP control diet group was significantly lower ( $P<0.05$ ) than that on the High-CP control diet group (Figure 2).

### 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. On the same level of the CP, enzyme supplementation only had marginal effects, tending to increase weight except



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

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**Figure 2** Feed intake of pullets on different and at different phases of feeding.

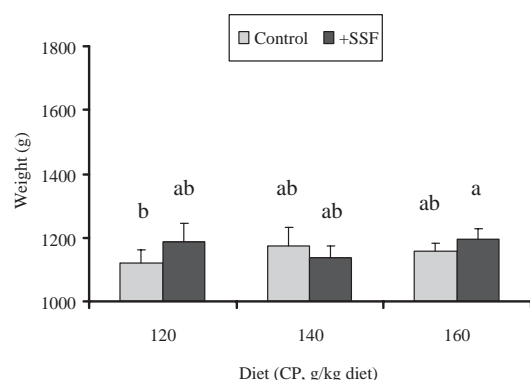
on the mid-CP diet. Body weight on the Low-CP control diet was lower than on the High-CP enzyme-supplemented diet ( $P<0.05$ ). There was no significant effect of CP, enzyme supplementation or their interaction on body weight at 20 weeks of age (Figure 3).

### 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 decreased on the Low- and High-CP diets but not on the Mid-CP diet, as a result of enzyme supplementation (Figure 4). Over the early period on the layer diet (16-20 weeks of age) and over the entire trial period, the enzyme supplement tended to decrease FCR, but this effect was not significant.

### Ovary weight and egg production:

By supplementation of microbial enzyme at the low CP content found the improvement of ovary weight compare with the treatment without adding enzyme to the diet ( $P<0.05$ ) (Figure 5). There was no significant effect of dietary protein content except between the Low-CP control and High-CP enzyme-supplemented diets, pullets on

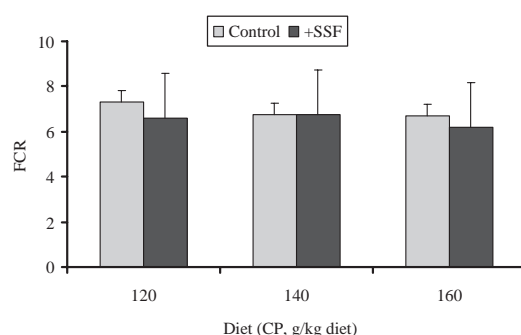


a,b – Mean values not sharing a superscript are significantly different ( $P<0.05$ ).

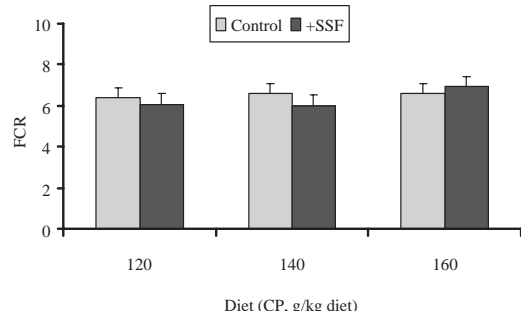
**Figure 3** Body weight of pullets at 16 and 20 weeks of age.

the latter having significantly heavier ovaries than the former ( $P<0.05$ ). 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.

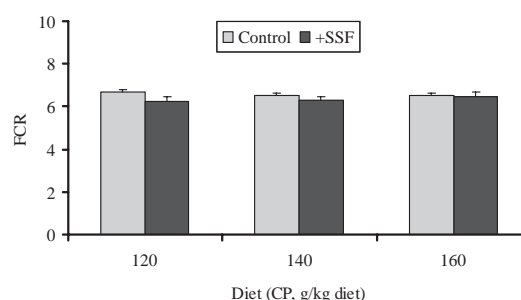
### (a) 12-16 weeks



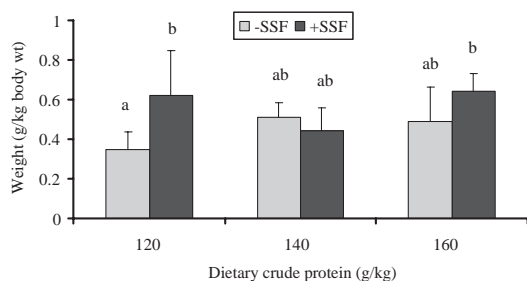
### (b) 16-20 weeks



### (c) 12-20 weeks



**Figure 4** Feed conversion ratio (FCR) of pullets on different diets at different phases of feeding.



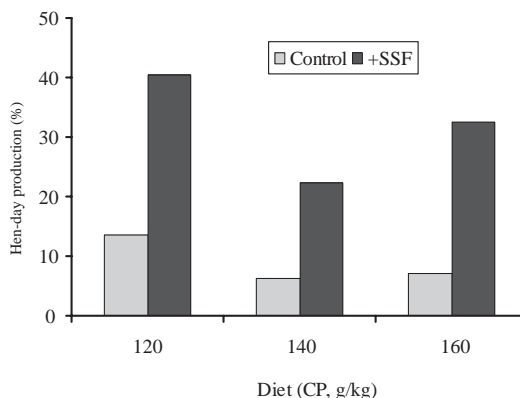
a,b – Mean values not sharing a superscript are significantly different ( $P < 0.05$ ).

**Figure 5** Ovary weight (g/kg body weight) at 18 weeks of age.

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 at 20 weeks of age, showed that egg production was much improved on the SSF-supplemented diets group, but more so on the Low-CP diet (Figure 6). At 23 weeks of age, hen-day egg production was about 13.5%, 40.5%, 6.3%, 22.2%, 7.2% and 32.5 % in groups the Low-CP control, Low-CP enzyme-supplemented, Mid-CP control, Mid-CP enzyme-supplemented, High-CP control and High-CP enzyme-supplemented diets, respectively.

## DISCUSSION

The current results demonstrated that feed intake, body weight and FCR at any level of dietary protein were 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



**Figure 6** Hen-day egg productions at 23 weeks of age.

supplement tended to reduce feed conversion ratio, probably as result of better feed utilization. The better 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 in broiler diet based on copra meal with the inclusion of Allzyme SSF® significantly increased feed conversion efficiency, nutrient digestibility and decreased jejunal content viscosity.

The data from the current trial showed that the protein level in the diet affected ovary weight and egg production. Low-protein diets can result in a reduction in egg production. Soares *et al.* (2003) suggested that high quality of protein with adequate amino acid balance was 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. The digestibility of the diets was also improved by adding enzyme supplementation. Ishibashi and Yonemochi (2003) also suggested that the availability of amino acid in the diet may be improved by feed management, especially supplementation with the microbial enzyme.

## CONCLUSION

The results demonstrate the negative effects of low dietary protein content on body weight and ovary weight. Supplementation with the microbial enzyme at the pullet stage, however, was shown to improve egg production later in life.

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