



## Effect of supplementing CLOSTAT® to gestating and lactating sows on performance, oxidative status and microbiome

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### Key Conclusions

- Inclusion of CLOSTAT on sows diets during late gestation and lactation resulted in increased number of piglets born alive, shortened piglet birth intervals, improved oxidative stress status at farrowing and improved piglet growth
- CLOSTAT had a significant positive impact on the intestinal microbiome, reducing the relative abundances of the Phyla Proteobacteria and Streptococcus

### INTRODUCTION

Increasing litter size in modern sow genetics poses a challenge to the sow in late gestation and lactation. At these stages sows face changes in management, feeding and housing but also physiological changes as they face inflammatory processes peripartum. There are also intestinal changes that may lead to undesirable bacterial proliferation, which may result in the production of endotoxins. Absorption of these will put further challenges on the health status of the sow, as they can lead to inflammation creating even more pressure on the oxidative stress balance. Farrowing duration is also a risk factor, with increased farrowing duration being associated with increased number of piglets born dead or hypoxic as well as post farrowing complications.

The objective of this study was to investigate the effects of supplementing sows feed from 90 days of pregnancy to 21 days after farrowing with CLOSTAT® on the reproductive performance, oxidative stress status, piglet performance during lactation and sows intestinal microbiome.

### KEYWORDS

CLOSTAT®, sow, piglets, lactation, microbiome, performance, oxidative stress, microbiome

## MATERIAL AND METHODS

The trial was carried out in a commercial farm in the Sichuan province, China. A total of 32 (LWxLR) sows with an average parity of  $2.47 \pm 0.5$  were included in the study.

At the day 90 of gestation sows were randomly allocated to 1 of 2 groups, with sows in the control group receiving the basal gestation and lactation diet and sows in the CLOSTAT group receiving the basal gestation (from day 90 of gestation to day 110 of gestation) or lactation (from day 110 of gestation to day 21 of lactation) diets supplemented with *Bacillus sp.* PB6 (CLOSTAT, Kemin) @  $4.0 \times 10^8$  CFU /kg feed. A summary of the treatments is available in Table 1. A feed curve was followed during lactation and weaning was carried out at day 21 of lactation.

Table 1. Summary of the two treatment groups

	Treatment
Control	Basal diet
CLOSTAT group	Basal diet + <i>Bacillus sp.</i> PB6 @ $4.0 \times 10^8$ CFU /kg feed

Within the first 24 h after farrowing, the litters were standardized to approximately 12 piglets per sow, the cross fostering was carried out between animals within the same treatment.

At farrowing, litter size, stillbirths, born alive, stillborn, piglets birthweights, duration of farrowing and feed used was recorded.

Blood samples were collected from the sows at day 0 (farrowing), day 14 and day 21 and from the piglets at 14 and 21 days of age. Fresh feces (n=5 / treatment) were collected on day 110 of gestation.

Blood samples (n=6 / treatment) were tested to assess the total antioxidant capacity (T-AOC), malondialdehyde which is a marker of oxidative stress (MDA), endotoxin concentrations (toxins produced by bacteria or resulting from bacterial death) in the sow and cortisol concentrations as a marker for stress response in the piglet sera. Fecal samples DNA was extracted and sent for 16S rRNA V4 amplicon sequencing for microbiome analysis.

## RESULTS AND DISCUSSION

### Reproductive performance of sows at farrowing

Supplementation of the sows gestation diet with CLOSTAT resulted in increased litter sizes (total born) and numbers of piglets born alive ( $P < 0.01$ ). Farrowing duration tended to be shorter with CLOSTAT supplementation, as a result of shorter period between piglets birth ( $P=0.022$ ), a summary of the farrowing performance results for the two treatments can be seen on Table 2.

Table 2. Summary of the reproductive performance of sows at farrowing

	CLOSTAT	Control	P - value
Average litter size (n=)	14.94 <sup>a</sup> ±0.36	13.13 <sup>b</sup> ±0.53	< 0.01
Average piglets born alive (n=)	14.00 <sup>a</sup> ±0.32	11.63 <sup>b</sup> ±0.64	< 0.01
Born alive (%)	93.97 ±1.66	88.29 ±2.89	0.098
Farrowing length (min)	218.81 ±8.54	255.6 ±18.98	0.092
Piglet birth interval (min)	15.03 <sup>b</sup> ±0.58	21.65 <sup>a</sup> ±2.55	0.022

### Lactation performance

Average litter size, average piglet weight at weaning, litter weight gain and pre-weaning mortality, were significantly improved in the CLOSTAT group, while there was no difference in the average sow feed intake between both groups. A summary of the sows performance during lactation can be found on Table 3.

Table 3. Summary of the sows performance during lactation

	CLOSTAT	Control	P - value
Litter size at cross fostering (n=)	12.31 ±0.22	12.31 ±0.33	1.00
Litter size at weaning (n=)	10.88 <sup>a</sup> ±0.2	9.56 <sup>b</sup> ±0.39	< 0.01
Pre-weaning mortality (%)	11.37% <sup>b</sup> ±1.99	21.87% <sup>a</sup> ±3.12	< 0.01
Average piglet body weight at cross fostering (kg)	1.40 <sup>b</sup> ±0.04	1.55 <sup>a</sup> ±0.05	0.026
Average piglet body weight at weaning (kg)	6.37 ±0.15	6.03 ±0.30	0.331
Average sow feed intake (kg/d)	5.76 ±0.10	5.77 ±0.15	0.956

### Oxidative Status

The serum concentration of MDA was significantly lower ( $P=0.004$ ) at farrowing on the CLOSTAT treated group than on the Control group ( $4.07 \pm 0.27$  and  $7.16 \pm 0.77$  respectively), no significant differences were observed between the two groups at the other time points (days 14 and 21 of lactation), also at farrowing the T-AOC was significantly increased ( $P=0.044$ ) on the sows treated with CLOSTAT ( $15.68 \pm 2.65$  U/ml) compared to sows on the Control group ( $8.65 \pm 1.51$ ). No significant differences were observed at the other time points (d 14 or d21 of lactation).

### Effect of treatments on serum endotoxin and cortisol levels

Serum endotoxin concentrations were significantly lower at 14 days of lactation ( $P = 0.007$ ) and showed a tendency to be lower at 21 days of lactation ( $P=0.059$ ) in the CLOSTAT group than in the Control group.

The piglets from sows included in the CLOSTAT group had significantly lower serum concentrations of cortisol at 14 days of age than piglets from sows included in the Control group ( $P =0.042$ ), no significant difference was observed at 21 days of age.

### Effect of treatments on sow microbiome

Supplementation with CLOSTAT from day 90 to 110 of gestation resulted in a significant increase in the relative abundance of Gemmatimonadete ( $P=0.001$ ) and Acidobacteria ( $P=0.003$ ) and a significant reduction of the relative abundance of Proteobacteria, which includes important pathogenic bacteria such as *Escherichia coli*, *Salmonella enterica*, *Yersinia enterocolitica* ( $\gamma$ -Proteobacteria), *Desulfovibrio piger* ( $\delta$ -Proteobacteria) and *Campylobacter jejuni* ( $\epsilon$ -Proteobacteria). ( $P=0.017$ ) and Actinobacteria ( $P=0.004$ ). The CLOSTAT group had a significant increase in the relative abundance of *Ruminococcaceae* ( $P=0,040$ ) and significantly reduced relative abundance of *Streptococcus* ( $P=0.030$ ).

## CONCLUSION

In conclusion, in the conditions of this trial, CLOSTAT have shown to be a valuable tool in supporting sow performance when supplemented during late gestation and lactation. Supplementation of late sow gestation diet and lactation diet resulted in improved farrowing and lactation performance. The sows supplemented with CLOSTAT had a better oxidative stress status than sows in the Control group, with a lower concentration of MDA (a marker for oxidative stress) and a higher T-AOC. Supplementation with CLOSTAT also resulted in less endotoxins in the sows sera and less cortisol in piglets indicating a lower stress level of the piglets during lactation. CLOSTAT also had a positive impact on the sows microbiome, with a reduction of relative abundance of Proteobacteria (which includes many pathogenic bacteria) at a phylum level and *Streptococcus* at a genus level (members of this genus are commonly associated with meningitis in pigs) and an increase on the relative abundance of *Ruminococcaceae* a family that includes beneficial butyrate producing species.

This study demonstrates that CLOSTAT is a valuable tool to support sow performance during farrowing and lactation but also to improve piglets early life survivability and weight, with lifelong benefits.

## REFERENCES

1. Zhang Q. et al. (2020). Dietary supplementation of *Bacillus subtilis* PB6 improves sow reproductive performance and reduces piglet birth intervals. *Animal Nutrition* 6, 278-287