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Research Article

Supplementation of a β-Mannanase Enzyme to Diets with a Reduced Net Energy Content Supports Post-Weaning Piglet Performance During a PRRSV Outbreak Under Field Conditions

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Abstract

β - Mannans - strongly anti-nutritive polysaccharide fibers - are found in many vegetable feed ingredients. In common swine diets, the content of soluble β-mannans is estimated to range between 0.15 to 0.40%. In vitro studies have demonstrated that as little as 0.05% of soluble β-mannan content in feed can elicit a strong innate immune response. Hemicell HT (Elanco Animal He alth) is a β-mannanase enzyme to supplement animal feed which breaks down β-mannans. Hemicell HT minimizes production and economic losses caused by the wasteful Feed-Induced Immune Response (FIIR) elicited by β-mannans. This field study compared pig performance on a control diet to a reformulated diet with lower energy content - 55-65 kcal/kg Net Energy (NE) reduction - including a β-mannanase enzyme during a PRRSV outbreak under field conditions. A six-week feeding trial was conducted on a commercial post-weaning facility with DanBred x Belgian Piétrain pigs starting at 21 days of age. Standard three-phase control diets were compared to reformulated diets with an energy reduction of 55-65 kcal NE/kg and inclusion of a β-mannanase enzyme (Hemicell HT™; Elanco) at 300g/tonne. Standard production data were collected. The data were analyzed using JMP 15.0 statistical program. Overall, performance data did not differ significantly between treatment groups during the post-weaning period. Mortality was only numerically, but not significantly higher in the Control as compared to the Enzyme-treated group. The effect of Enzyme supplementation was beneficial in both light-weight and heavy-weight piglets to maintain performance during a PRRSV outbreak. Hemicell HT had an overall benefit of € 3.59 per piglet and € 5.18 per tonne of feed due to the NE reduction. The current trial demonstrated that the inclusion of Hemicell HT in reformulated diets with a lower energy content (55-65 kcal NE/kg) was able to retain production performance in post-weaned piglets during a PRRSV outbreak.

Keywords: β - Mannanase, Post-weaned pigs, Net energy reduction, PRRSV outbreak, Economic benefit

Abbreviations: ADFI: Average Daily Feed Intake; ADWG: Average Daily Weight Gain; FCR: Feed Conversion Rate; FIIR: Feed Induced Immune Response; NE: Net Energy; NSP: Non-Starch Polysaccharide; PAMP: Pathogen Associated Molecular Pattern; PRR: Pathogen Recognition Receptors; PRRSV: Porcine Reproductive and Respiratory Syndrome Virus; PCV-2: Porcine Circovirus type 2; PWD: Post-Weaning Diarrhea; SBM: Soybean Meal

Introduction

It is estimated that by 2031, pig meat consumption will represent 27.9% of all meat protein sources worldwide, with an estimated increase in consumption of 2.4% compared to 2019-2021 [1]. One of the major problems now and in the future is to retain production costs at an acceptable economic level. Currently, the feed costs form an important part of the total cost in swine production (>60 %) with energy accounting for at least 70% of feed costs. Therefore, feed additives that can improve overall feed utilization are very important for the swine industry. Polysaccharides, polymers of monosaccharides linked by glycosidic bonds, are major components of all vegetable feed ingredients used in common swine diets. Components such as starch are digested in the small intestine of pigs through endogenous enzyme activity. However, Non-Starch Polysaccharides (NSPs), fibrous materials found in the plant cell wall, which include celluloses, hemicelluloses, pectins, and oligosaccharides, are more difficult to digest, since monogastric animals - such as pigs - do not produce the endogenous enzymes needed to digest β-linked NSPs [2]. β - Mannan - an anti-nutritive factor found in many common feed ingredients - has been intensively studied in recent years [3]. β - Mannans are linear polysaccharides constituting a linear backbone on which repeating units of β-1,4-mannose and α-1,6-galactose and/or glucose units are attached [4,5]. In monogastric diets, high concentrations of β - mannans have distinct anti-nutritive properties, mainly due to stimulation of the innate immune response. Pathogen-Associated Molecular Patterns (PAMP), which include complex polysaccharides that resemble β - mannans, are distinct molecules expressed on the pathogen surface that bind to the Pathogen Recognition Receptors (PRR) present on the cell surface of innate immune cells [6]. Connection between PAMP and PRR results in the release of innate defence molecules such as complement proteins, antimicrobial peptides, bacteriolytic enzymes and reactive oxygen or nitrogen species [7]. Thus, β-mannans present in the gastro-intestinal tract through the feed can be mistaken for an invading pathogen by the immune system subsequently causing an unwarranted immune activation [8,9], which has been called a Feed-Induced Immune Response (FIIR) [10]. This immune reaction, provoked by the misrecognition of β-mannans as an invading pathogen, causes energy and nutrients to be wasted [4]. Therefore, hydrolysis of these β-mannans through the inclusion of exogenous β-mannanase enzymes can reduce and potentially eliminate their ability to induce a FIIR.

Recent studies have demonstrated positive effects of supplementing β-mannanase to various swine diets on nutrient digestibility and growth performance [11-13]. Additionally, supplementation of β-mannanase to corn-SBM diets was able to reduce the population of faecal coliforms and the NH3 concentration of faecal slurry after 24h fermentation [14], which might impact the environmental infection pressure from coliforms, related to clinical problems of Post-Weaning Diarrhoea (PWD) [15]. Therefore, supplementation of a β-mannanase enzyme to post-weaning diets can reduce or eliminate the occurrence of FIIR and increase available energy and proteins for growth. Based on these assumptions, several field studies reducing the available dietary NE by 45 to 65kcal per kg feed in both nursery [16-17] and fattening diets [18,19] demonstrated similar performance with decreased production costs.

The objective of the current study was to evaluate the effects of β-mannanase supplementation to post-weaning diets with a reduced NE content of 65kcal/ kg in Phase 1-2 and 55kcal/kg in Phase 3 on piglet performance and economic parameters during a PRRSV outbreak under field conditions in the post-weaning phase.

Materials and Methods

Description of Experimental Farm

The post-weaning field trial was performed on a conventional farrow-to-finish swine herd in Belgium with 2 compartments with 24 pens each. Each pen housed 32 post-weaned piglets. Piglets were equally distributed to the Control group (n=24 pens, 748 piglets) and the Enzyme-treated group (n=24 pens, 765 piglets). Compartments were ventilated through mechanical ventilation with an air inlet through the door. All pens had partially slatted plastic floors. Water was distributed through a nipple in the feeder. Each pen was equipped with one dry feeder. Meal feed consumption was registered at group level. Both study groups were randomly distributed within both post-weaning compartments.

Experimental Design

Treatment Groups: At weaning the piglets were assigned to one of both treatment groups, Control and Enzyme-treated, respectively. A three-phase diet was distributed with Phase 1 during week 1-2, Phase 2 during week 3-4 and Phase 3 during week 5-6 (Table 1). Groups were blinded to the farm personnel and only distinguished by color codes (red, Enzyme-treated group; blue, Control group). Piglets from each individual pen were considered one experimental unit and were weighed together.

Table 1: Feed price (€/tonne of feed), net energy content (kcal/kg), β-mannan content (%) and feed composition changes from a feed trial with a 3-phase feeding strategy comparing standard Control diets to adapted Enzyme-treated diets.

Feed Phase	Phase 1		Phase 2		Phase 3	
	Post- Weaning Starter		Starter		Growth Starter	
Treatment	Control	Enzyme	Control	Enzyme	Control	Enzyme
Feed price (€/ tonne)	619	617.5	551	549	511	506
Net energy content (kcal/kg)	2,400	2,335	2,390	2,325	2,410	2,355
Δ Net energy content (kcal/kg)		65		65		55
β-mannan content (%)	0.343	0.344	0.339	0.339	0.354	0.361
Composition Changes						
Wheat					20.1	22.6
Cookie mix					4	2
Soy concentrate	4	2	4	2		
Soybean meal 49	1.5	4	6.6	8.8		
Soy oil					2.38	1.65

Experimental Diets: The pigs were fed a three-phase mash diet consisting of phase 1 (0-14 d), phase 2 (15-28 d), and phase 3 (29-44 d) in each of the treatment groups. The main difference between the diets in Control and Enzyme-treated group was a reduction in NE content of 65, 65, and 55 kcal/kg feed in Phase 1, 2, and 3, respectively (Table 1) . The Enzyme-treated group was supplemented with a β-mannanase enzyme (Hemicell™ HT; Elanco, Indianapolis; IN) at an inclusion rate of 300g per tonne of feed, according to the manufacturer's instructions for use. All other enzymes (xylanase and phytase) in the diets remained at the same levels in both study groups.

Experimental Animals: DanBred * Belgian Piétrain piglets were obtained from the conventional commercial sow farm linked to the post-weaning facility. Piglets were vaccinated to protect against *Mycoplasma hyopneumoniae* and Porcine Circovirus type 2 (PCV-2) using a one-shot commercial vaccine (Ingelvac Combo-Flex; Boehringer Ingelheim). One single batch of piglets (n=1504) was enrolled for the feed trial.

Health Status of the Herd and Enrolled Piglets

The conventional sow farm was negative for Aujeszky disease (Pseudorabies virus), Brucellosis, Classical Swine Fever, African Swine Fever, and all types of *Brachyspira* species. The farm was confirmed positive for *M. hyopneumoniae*, Porcine Reproductive and Respiratory Syndrome virus (PRRSV), and PCV-2. The batch of piglets enrolled in the post-weaning field trial suffered from confirmed circulation with PRRSV and had secondary disease issues related to *Streptococcus suis*. Clinical symptoms related to PWD due to enterotoxigenic *E. coli* strains were limited.

Performance Data Collection

Pig Body Weight (BW) per pen was measured at 0- and 44-days post-weaning. Feed provision (*ad libitum*) was only recorded at treatment group level. Average Daily Weight Gain (ADWG; expressed as g/d), Average Daily Feed Intake (ADFI; expressed as g/d) and Feed Conversion Rate (FCR; expressed as kg feed per kg weight gain) were calculated. Mortality was recorded with date of death, weight, and number of dead animals.

Veterinary Treatments

Individual antibiotic treatments were performed as needed due to the critical state of the piglet and in case of a broader health issue in the barn, group treatment could be performed. The same veterinary products and dosages (ml/kg) were used throughout the entire study period. Individual antimicrobial treatments or group treatments were recorded daily by date, product, dose, ID number of treated piglets, presumed cause of treatment, and number of times the treatment was repeated.

Economic Benefit Per Piglet and Per Tonne of Feed

The economic benefit of supplementation of β-mannanase combined with a reduction in the NE of 55-65 kcal/kg feed was calculated both at piglet level and at feed cost level. For the calculation of the economic benefit at piglet level, the following parameters were considered: feed cost reduction, piglet price correction (standard price for 25kg piglet during the trial period was at € 75), and opportunity costs of mortality. For the calculation of the economic benefit at feed cost level, the following parameters were considered: the total feed cost and the total amount of feed consumed.

Data Management and Statistical Analysis

Data were hand-recorded by the farm personnel and stored in MS Excel on OneDrive at the end of each day. Following the end of the feed trial, data were extracted from Excel into JMP 15.0, and the blinded color-coded treatments were unblinded to reveal the respective treatment groups. Calculations, exploratory data analysis and quality review, and subsequent statistical analysis were all performed in JMP 15.0. All data are presented as means with their respective pooled Standard Error of the Mean (SEM). All means were tested for significant differences (P<0.05) using a T-test.

Results

Pig Weight and Average Daily Weight Gain

Data on pig weight is given in Table 2. The piglets were transferred to the post-weaning facility at an average weight of 5.1kg. No significant differences (P>0.05) were present in the start weight (d0) between both treatment groups. At d44, the end of the post-weaning feed trial, the piglets in the Enzyme-treated group were again slightly, but not significantly (P>0.05) heavier with 20.0 kg (±0.8) as compared to the Control group (19.2±0.9kg). When analyzed based on weight categorization Table 3, the light-weight piglets (25% pens with the lightest piglets) in the Control group were numerically, but not significantly (P > 0.05), lighter (14.9 kg±0.8) as compared to the piglets in the Enzyme-treated group (15.8 kg±0.7). The medium-weight piglets (50% pens with intermediate piglets) in the Control group were again numerically, but not significantly (P > 0.05), lighter (19.1 kg±0.6) as compared to the piglets in the Enzyme-treated group (20.1 kg±0.6). The heavy-weight piglets (25% pens with the heaviest piglets) in the Control group were only slightly (P > 0.05) lighter (23.6 kg±0.2) as compared to the piglets in the Enzyme-treated group (24.0 kg±0.2) (Table 2).

Table 2: Summary of performance data from a feed trial with a 3-phase feeding strategy comparing standard Control diets to adapted Enzyme-treated diets.

	Control	Hemicell HT	P-value
# pens	24	24	-
Total # piglets d0	737	765	-
Total # piglets d44	709	736	-
Mortality (#)	39	29	0.23
Mortality (%)	5.3±1.4	3.8±1.3	0.19
Weight d0 (kg)	5.1±0.3	5.1±0.2	0.48
Weight d44 (kg)	19.2±0.9	20.0±0.8	0.19
Weight dead piglets (kg)	10.3±2.0	7.8±2.1	0.2
ADWG (g/d)	315±14	333±14	0.18
ADFI (g/d)	492	498	-
FCR (kg/kg gain)	1.53	1.48	-
Total feed (tonne)	15,725	16,485	-
Total feed cost (€)	8615.48	8946.36	-
Feed cost (€/piglet sold)	12.15	12.16	-
Feed cost per kg gain (€/kg)	0.861	0.82	-

Data on ADWG is also given in Table 2. Overall, ADWG was not significantly different between both study groups (333 g/d±14 vs. 315 g/d±14 in Enzyme-treated and Control group, respectively). When analyzed based on weight categorization Table 3, ADWG of the light-weight piglets (25% pens with the lightest piglets) in the Control group was numerically, but not significantly (P>0.05), lower (248 g/d±13) as compared to the piglets in the Enzyme-treated group (259 g/d±13). The ADWG of the medium-weight piglets (50% pens with intermediate piglets) in the Control group was again numerically, but not significantly (P>0.05), lighter (312 g/d±10) as compared to the piglets in the Enzyme-treated group (338 g/d±10). The ADWG of the heavy-weight piglets (25% pens with the heaviest piglets) in the Control group was also numerically, but not significantly (P>0.05), lighter (385 g/d±2) as compared to the piglets in the Enzyme-treated group (397 g/d±3) (Table 3).

Table 3: Summary of performance data from the 25% light-weight pens (n=6), 50% medium-weight pens (n=12), and the remaining 25% heavy-weight pens (n=6) from a feed trial with a 3-phase feeding strategy comparing standard Control diets to adapted Enzyme-treated diets.

	Control	Hemicell HT	P-value
Light-Weight pens (25%)			
# pens	6	6	-
Total # piglets d0	160	188	-
Total # piglets d44	153	176	-
Mortality (#)	18	12	0.25
Mortality (%)	11.1±2.0	6.8±1.4	0.19
Weight d0 (kg)	4.1±0.4	3.8±0.2	0.36
Weight d44 (kg)	14.9±0.8	15.8±0.7	0.38
Weight dead piglets (kg)	12.8±2.2	8.5±1.2	0.20
ADWG (g/d)	248±13	259±13	0.39
Medium-Weight pens (50%)			
# pens	12	12	-
Total # piglets d0	385	385	-
Total # piglets d44	369	369	-
Mortality (#)	16	16	0.50
Mortality (%)	4.1±0.1	4.1±0.1	0.49
Weight d0 (kg)	5.1±0.2	5.1±0.2	0.49
Weight d44 (kg)	19.1±0.6	20.1±0.6	0.18
Weight dead piglets (kg)	8.7±2.0	9.7±2.6	0.41
ADWG (g/d)	312±10	338±10	0.11
Heavy-Weight pens (25%)			
#pens	6	6	-
Total # piglets d0	192	192	-
Total # piglets d44	556	560	-
Mortality (#)	5	1	0.04
Mortality (%)	2.6±0.5	0.5±0.3	0.04
Weight d0 (kg)	6.5±0.1	6.5±0.1	0.42
Weight d44 (kg)	23.6±0.2	24.0±0.2	0.24
Weight dead piglets (kg)	10.8±2.2	3.2±1.6	0.09
ADWG (g/d)	385±2	397±3	0.08

Average Daily Feed Intake and Feed Conversion Rate

Data on ADFI and FCR is given in Table 2. Overall, ADFI was 6 g/d higher in the Enzyme-treated group (498 g/d) as compared to the Control group (492 g/d). Overall, FCR was 0.05 lower in the Enzyme-treated group (1.48 kg feed/kg weight gain) as compared to the Control group (1.53 kg feed/kg weight gain).

Antimicrobial Treatment

No significant differences were observed neither at the level of individual treatment nor group treatment between both treatment groups during the entire post-weaning feed trial.

Mortality

Data on mortality is given in Table 2. Numerically, a total of 29 piglets died in the Enzyme-treated group as compared to 39 in the Control group over the entire study period. Overall, mortality was slightly, but not significantly (P>0.05) lower (3.8 %±1.3) in the Enzyme-treated group as compared to the Control group (5.3 %±1.4). When analyzed based on weight categorization Table 3, a total of 12 piglets died in the light-weight category of Enzyme-treated pigs, whereas 18 died in the Control pigs. Therefore, mortality was much higher (P>0.05) in the Control group (11.1 %±2.0) as compared to the Enzyme-treated group (6.8 %±1.4). In the medium-weight pi-

glets, mortality was equal (n=16; 4.1 %) in both treatment groups. In the heavy-weight piglets, a significantly lower (P < 0.05) mortality was observed in the Enzyme-treated group (n=1; 0.5 %) as compared to the Control group (n=5; 2.6 %).

Economic Benefit Per Piglet and Per Tonne of Feed

The detailed calculation of economic benefit per piglet is given in Table 4. Overall, supplementation of a β-mannanase enzyme combined with a reduction of NE with 65, 65, and 55 kcal/kg feed over the three phases, respectively, resulted in an economic benefit per piglet of € 3.59. The detailed calculation of economic benefit per tonne of feed is given in Table 4. Overall, supplementation of a β-mannanase enzyme combined with a reduction of NE with 65, 65, and 55 kcal/kg feed over the three phases, respectively, resulted in a feed cost reduction of € 5.18 per tonne of feed (Table 4).

Table 4: Detailed calculation of economic benefit per piglet considering the reduction in feed cost, piglet price corrections (standard price at 25kg) and opportunity cost of mortality.

Parameter	Control	Hemicell HT
Feed cost per piglet (0-42 d)	€ 12.15	€ 12.16
Benefit feed cost reduction		-€ 0.01
Piglet price corrections (€ 75, - for 25kg)	-€ 17.40	-€ 15.00
Benefit technical results		+€ 2.40
Mortality (#)	39	29
Total opportunity cost due to mortality (€)	€ 2,925	€ 2,175
Opportunity cost per marketed piglet (€/piglet)	€ 4.12	€ 2.92
Benefits mortality		+€ 1.20
Overall benefit per piglet		+€ 3.59

Discussion

In the current field study, the β-mannan content in all phases ranged from 0.339 to 0.361% and was therefore sufficiently high to assure efficient activity for the supplemented β-mannanase. Moreover, the standard feed composition could be used in the trial without the need for additional substitutions of more expensive proteins to extruded SBM, as previously reported [16]. The current level of β-mannans, which are known as anti-nutritive factors [3], may induce an innate immune response as they resemble PAMPs [6] and therefore lead to a FIIR (Feed Induced Immune Response; [10]). This induces an unnecessary immune activation which causes energy and nutrients to be wasted [4]. To hydrolyze the anti-nutritive β-mannans in the trial feed, 300g/tonne of an exogenous β-mannanase enzyme (Hemicell HT; Elanco, Greenfield, IA) was added to the composed feed. The enzyme supplementation should result in a reduction of futile immune activation due to FIIR and therefore, the spared energy was compensated by a reduction of 55-65 kcal NE per kg feed.

The performance results demonstrated no significant differences in the measured (piglet weight, ADFI) or calculated (ADWG, FCR) performance parameters between both treatments. This confirmed that the addition of an exogenous β-mannanase to adapted formulations with a reduction in NE content of 55-65 kcal per kg feed, in the presence of a sufficient level of β-mannans, allowed to perform equally to the standard post-weaning Control diets. The obtained results correspond to other studies with dietary changes and the addition of a β-mannanase [13,16-18].

The observed mortality in both study groups was rather high according to industry standards and historical farm records. This could be attributed to an active PRRSV circulation in the post-weaning phase, as confirmed by PCR, and secondary complications due to *S. suis* meningitis. Therefore, the data of the 24 pens per treatment group were broken down into three different weight categories - light - weight (n=6), medium-weight (n=12) and heavy-weight (n=6) piglets - to analyze the performance data separately to identify any beneficial effects of the supplemented β-mannanase on piglets suffering a PRRSV outbreak under field conditions. Although numerical improvements could be observed in the Enzyme-treated group, only mortality in the heavy-weight piglets was significantly better as compared to the Control group. In the heavy-weight piglets, a trend (P<0.10) was present for the weight of dead piglets and ADWG in the Enzyme-treated group. Therefore, supplementation of β-mannanase under conditions of a PRRSV outbreak does not particularly support piglets in specific weight categories but retains overall performance with diets formulated with a 55-65 kcal NE reduction.

Although performance results remained at the same level in both treatment groups, a substantial economic benefit of β-mannanase enzyme supplementation could be calculated. Based on the feed prices Table 1 and the actual feed consumed Table 3, a 1 % reduction (€ 5.18) in feed cost per tonne of feed (€ 542.70 vs. € 547.88, in Enzyme-treated vs. Control group, respectively) could be obtained. Considering all costs and income related to piglet production, including feed cost, opportunity costs for mortality and

piglet base market price at 25 kg, the income per produced piglet was € 3.59 higher for piglets in the Enzyme-treated group.

Others concluded that supplementation of a β-mannanase enzyme could improve growth performance in both weanling and growing-finishing pigs on corn-SBM diets [11,12]. A diet with a 150 kcal/kg reduction in digestible energy supplemented with β-mannanase outperformed in weight gain and feed efficiency [11]. Others have also observed the energy-sparing effect from the supplementation of β-mannanase. For example, the supplementation to a common nursery diet resulted in similar effects on performance of a comparable diet supplemented with 2% soya oil [12]. In poultry, beneficial effects of β-mannanase supplementation on the performance of chickens challenged with *Eimeria* sp. and *Clostridium perfringens* were observed together with reduced lesion scores in disease challenged birds [20]. This observation was confirmed by a recent study in post-weaned piglets, where antimicrobial use for the treatment of PWD due to *E. coli* was significantly reduced in the Enzyme-treated group as compared to the Control group [15]. However, in the current study, the disease outbreak during the post-weaning period was related to PRRSV and secondary *S. suis* and we could not observe any differences in antimicrobial treatment between both treatment groups. Nevertheless, supplementation of a β-mannanase combined with a reduction in NE content could maintain production performance in all economically important parameters at the same level of the Control group (Table 5).

Table 5: Detailed calculation of economic benefit of feed cost per tonne of feed considering total feed costs and total amount of feed consumed.

Parameter	Control	Hemicell HT
Total feed costs (0-44 d)	€ 8,615.48	€ 8,946,36
Total amount of feed consumed (tonne)	15,725	16,485
Feed cost per unit (€/tonne)	€ 547.88	€ 542.70
Overall benefit per tonne of feed		-€ 5.18

Conclusion

The current trial demonstrated that the inclusion of Hemicell HT in reformulated diets with a lower energy content (55-65kcal NE/kg of feed) was able to maintain production performance in post-weaned piglets, suffering from an active PRRSV circulation and secondary *S. suis* meningitis, with an economic benefit. The inclusion of Hemicell HT had an overall benefit of € 3.59 per piglet and € 5.18 per tonne of feed due to the 55-65kcal/kg NE reduction.

Ethics Approval and Consent to Participate

Field trial with an EFSA approved feed supplement for use in swine. No additional ethical approval needed. Consent to participate was obtained following full information of the farmer on the protocol to be carried out.

Consent for Publication

Not applicable.

Author's Information

FV is currently a Principal Technical Advisor Swine & Nutritional Health for Benelux / UK&ROI within Elanco Animal Health. He holds a DVM, a master's in veterinary public health and food Safety, a PhD in Veterinary Sciences, a PhD in Applied Biological Sciences and an EBVSTM European Specialist in Porcine Health Management. He is a resident in the American Board of Veterinary Practitioners-Swine Health Management and has a particular interest in swine intestinal health and specific approaches to improve intestinal health through non-antibiotic solutions.

Author's Contributions

FV and AdB were both involved in study design, data collection, data analysis, and manuscript preparation. GV and PJ were involved in study design, feed formulation, trial follow-up, and data analysis. All authors read and approved the final manuscript.

Availability of Data and Material

The datasets analyzed during the current study are available from the corresponding author on reasonable request.

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Competing Interests

The authors declare that they have no other competing interests.

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