



Black wattle (*Acacia mearnsii*) condensed tannin extract as feed additive in diets of weaned piglets

Luciane Inês Schneider¹  Anderson Borba¹  Janaina Martins de Medeiros² 
Daniela Regina Klein¹  Bruna Poletti³  Carlos Augusto Rigon Rossi⁴ 
Alexandre de Mello Kessler³  Vladimir de Oliveira^{1*} 

¹Departamento de Zootecnia, Universidade Federal de Santa Maria (UFSM), 97105-900, Santa Maria, RS, Brasil. E-mail: vladimir.oliveira@ufsm.br.

*Corresponding author.

²Centro de Ciências Agroveterinárias, Universidade do Estado de Santa Catarina (UFSC), Lages, SC, Brasil.

³Departamento de Zootecnia, Universidade Federal do Rio Grande do Sul (UFRGS), Porto Alegre, RS, Brasil.

⁴Departamento de Clínica de Grandes Animais, Universidade Federal de Santa Maria (UFSM), Santa Maria, RS, Brasil.

ABSTRACT: This study evaluated the effect of black wattle (*Acacia mearnsii*) condensed tannin extract in simple and complex diets for weaned piglets on performance, faecal consistency and serum haptoglobin concentrations. Eighty-eight female and castrated male piglets were used, weaned at 28 ± 3 days old, with an initial body weight of 8.02 ± 1.21 kg, housed in same-sex pairs and distributed in four treatments in a 2×2 factorial experiment in a randomised complete block design. The experimental treatments were simple diet (SD), simple diet + tannin (SD+T), complex diet (CD) and complex diet + tannin (CD+T). The simple diets contained lower concentrations of dairy ingredients, inclusion of barley and no addition of zinc oxide, palatant, acidifier and yeast. The diets with tannin were supplemented with $1,850 \text{ mg kg}^{-1}$ of black wattle condensed tannin extract. The feed programme consisted of three diets: Pre-starter I (0–7 days), Pre-starter II (8–21 days) and Starter (22–28 days). Feed and water were available *ad libitum*. Body weight and feed intake were measured weekly to calculate the average daily feed intake, daily weight gain and feed conversion ratio. The faecal consistency score was measured daily to calculate the diarrhoea occurrence percentage. Blood samples for serum haptoglobin concentration were collected at 7 and 14 days after weaning. The addition of condensed tannin had no effect ($P > 0.05$) on evaluated performance variables for weaned piglets. The diet type also did not affect the piglets' performance ($P > 0.05$). The inclusion of black wattle (*Acacia mearnsii*) condensed tannin does not affect piglet performance, reduces the incidence of diarrhoea in the first week after weaning and decreases the haptoglobin inflammatory response.

Key words: black wattle tannin, feed additive, gut health, nutrition, post-weaning.

Tanino condensado de acácia-negra (*Acacia mearnsii*) como aditivo nutricional em dietas de leitões desmamados

RESUMO: O objetivo do estudo foi avaliar o efeito de extrato de tanino condensado de acácia-negra (*Acacia mearnsii*) no desempenho, consistência fecal e concentração sérica de haptoglobina em leitões na fase de creche. Foram utilizados 88 leitões desmamados aos 28 ± 3 dias, com peso vivo médio inicial $8,02 \pm 1,21$ kg, alojados em duplas de mesmo sexo, e distribuídos em quatro tratamentos em um delineamento de blocos inteiramente ao acaso, em esquema fatorial 2×2 . Os tratamentos experimentais foram: dieta simples (SD); dieta simples + tanino (SD+T); dieta complexa (CD) e dieta complexa + tanino (CD+T). As dietas simples foram formuladas com menores concentrações de derivados de leite, inclusão de cevada e sem adição de óxido de zinco, palatabilizante e levedura. As dietas com inclusão de tanino foram suplementadas com 1850 mg kg^{-1} de extrato concentrado de tanino de acácia negra. O programa alimentar foi constituído de três dietas: Pré-inicial I (0 a 7 dias), Pré-inicial II (8 a 21 dias) e Inicial (22 a 28 dias). Ração e água ficaram disponíveis *ad libitum*. O peso e o consumo de ração dos leitões foram mensurados semanalmente, para calcular a média diária de consumo, o ganho de peso diário e a conversão alimentar. O índice de consistência fecal foi medido diariamente para calcular a porcentagem de ocorrência de diarreia. Amostras de sangue para concentração sérica de haptoglobina foram coletadas aos sete e 14 dias após o desmame. A adição de tanino condensado não teve efeito ($P > 0,05$) no desempenho de leitões desmamados. O tipo de dieta também não afetou o desempenho dos leitões ($P > 0,05$). A inclusão do tanino condensado de acácia-negra (*Acacia mearnsii*) não afetou desempenho, reduziu a incidência de diarreia na primeira semana após o desmame, e diminuiu a resposta inflamatória da haptoglobina.

Palavras-chave: aditivo, leitões, nutrição, saúde intestinal, tanino de acácia-negra.

INTRODUCTION

The interest in the use of phytochemical feed additives to replace antimicrobials has grown in recent years, partly because of the need to adopt sustainable production strategies and to gain consumer acceptance (MAHFUZ et al., 2021). The use of sub-therapeutic doses of antibiotics in feed or antibiotic growth promoters (AGPs) in animal diets is being

linked to the development of bacterial resistance to antibiotic substances, affecting public health systems (MARSHALL & LEVY, 2011). Because of this, Europe has banned the use of AGPs in 2006, which has become a worldwide trend (STEINER & SYED, 2015). Phytochemical feed additives are commonly defined as plant bioactive substances, such as polyphenols, including tannins, with great potential in animal nutrition (STEINER & SYED, 2015).

For a long time, tannins were considered only as anti-nutritional factors, with detrimental effects on animal performance and growth (MARISCAL-LANDÍN et al., 2004). However, recent studies found that tannins have antiviral, insecticidal, antifungal, antibacterial and antioxidant properties (BARBEHENN & CONSTABEL, 2011; REGGI et al., 2020), in addition to anti-diarrhoeal effects (PALOMBO, 2006). The anti-inflammatory properties and the capacity to control pathogenic bacteria and modulate gut morphology justifies the use of phenolic compounds as feed additives in piglets diets (FIESEL et al., 2014; MAHFUZ et al., 2021) to substitute antibiotic growth promoters (MAHFUZ et al., 2021).

At weaning, piglets are subjected to nutritional, environmental and social stress (PLUSKE et al., 1996). These stressors result in low feed intake as well as changes in gut morphology and microbiota, which affect productive performance and increase susceptibility to diseases. The multiple functions of tannin polyphenols also have positive effects on gut microbiota (LAU et al., 2003), gut health and animal performance (BRUS et al., 2013), reducing post-weaning diarrhoea (LIU et al., 2020), and stress blood metabolites (CAPRARULO et al., 2020), which can revert weaning effects (HUSSAIN et al., 2021), thereby improving animal production and welfare.

In this context, the hypothesis was that the inclusion of black wattle condensed tannin in post-weaned piglet diets improves performance and gut health, contributing to reducing the use of antibiotic growth promoters. Condensed tannins are present in the feed dietary fibre fraction and have a tendency to form complexes with proteins; they also limit feed intake. In contrast, hydrolysable tannins are easily hydrolysed either spontaneously or by the action of enzymes. Condensed tannins are mostly used in ruminants and reduce the amount of protein digested in the rumen, increasing the protein available in the small intestine and eliminating parasites (MUELLER-HARVEY, 2006). In non-ruminants, the use of tannins has scarcely been studied, with emphasis on hydrolysable tannins. This study evaluated the effect of condensed tannin extract from black wattle (*Acacia mearnsii*) in post-weaning simple and complex diets on performance, faecal consistency and serum haptoglobin levels of piglets.

MATERIALS AND METHODS

The procedures adopted in this experiment followed the provisions of Federal Law Nº 11,794 of 8 October 2008 and Decree Nº 6,899 of 15 July

2009, under Case Nº 1888290720 of the local Ethics Committee of Animal Use (CEUA).

Animals, experimental design and installations

In total, 88 crossbred post-weaned piglets (44 females and 44 males), with an initial body weight of 8.02 ± 1.21 kg, were labelled and distributed in four treatments in a 2 x 2 factorial experiment in a randomized complete block design, with blocks defined by initial body weight and 11 repetitions.

The animals were housed in 44 pens (two animals of the same sex per pen) with an area of 1 m² and an elevated plastic floor, equipped with a stainless feeder and nipple drinker. The pens were in a masonry facility, with side curtains and exhaust fans to control ventilation and air renewal, equipped with air conditioners to maintain the temperature in the thermal comfort zone. A photoperiod of 12 hours of light was adopted. The facility was cleaned twice a day, removing faeces using water.

Diets and treatments

There were four diets used as treatments: simple diet (SD), simple diet + tannin (SD+T), complex diet (CD) and complex diet + tannin (CD+T). The dietary tannin supplementation of 1,850 mg kg⁻¹ was performed using 2.5 g kg⁻¹ of a commercial product containing 74% black wattle (*Acacia mearnsii*) condensed tannin extract and 26% soy lecithin.

The diets were formulated using the *FORMULAE*[®] software (OLIVEIRA et al., 2019), according to the nutritional recommendations of ROSTAGNO et al. (2017). The simple diets contained lower concentrations of dairy ingredients and had no addition of zinc oxide, acidifier and yeast compared to the complex diets (Tables 1 and 2).

The experimental period was 28 days, with a nutritional three-phase programme: Pre-starter I (0 to 7 days), Pre-starter II (8 to 21 days) and Starter (22 to 28 days). Feed and water were available *ad libitum*.

Performance, faecal consistency score and serum haptoglobin concentration

The piglets were weighed at the beginning of the study and at every 7 days until the end of the experiment after a previous fasting period of 12 hours to avoid weight changes because of gastrointestinal tract filling. Body weight was used to calculate the daily weight gain (DWG). The total feed intake (measured weekly) and eventual feed wasting were measured to calculate the average daily feed intake (ADFI). The feed conversion ratio (FCR) was calculated from the

Table 1 - Composition of experimental diets for Pre- starter I (1-7 days), Pre- starter II (8-22 days) and Starter (22-28 days) phases.

Ingredients (%)	-----PRE-STARTER I-----				-----PRE-STRATER II-----				-----STARTER-----			
	SD	SD+T	CD	CD+T	SD	SD+T	CD	CD+T	SD	SD+T	CD	CD+T
Corn	18.00	18.00	20.80	20.80	21.70	21.70	26.00	26.00	24.50	24.50	33.30	33.30
Rice meal	18.00	18.00	20.80	20.80	21.70	21.70	26.00	26.00	24.50	24.50	33.30	33.30
Barley	14.00	14.00	-	-	14.00	14.00	-	-	24.00	24.00	-	-
Soybean meal	22.00	22.00	20.00	20.00	23.00	23.00	21.00	21.00	29.00	29.00	26.50	26.50
Starch	0.25	-	0.25	-	0.25	-	0.25	-	0.25	-	0.25	-
Whey powder	15.00	15.00	15.00	15.00	8.00	8.00	8.00	8.00	-	-	-	-
Soy protein concentrate	-	-	5.00	5.00	-	-	3.00	3.00	-	-	-	-
Sugar	2.50	2.50	3.50	3.50	1.50	1.50	2.50	2.50	-	-	-	-
Spray dried blood plasma	2.50	2.50	3.50	3.50	2.00	2.00	3.00	3.00	-	-	-	-
Soybean oil	3.45	3.45	1.25	1.25	3.57	3.57	2.59	2.59	3.37	3.37	2.05	2.05
Dried milk	-	-	5.00	5.00	-	-	2.30	2.30	-	-	-	-
L-lysine	0.69	0.69	0.36	0.36	0.62	0.62	0.44	0.44	0.53	0.53	0.62	0.62
DL-methionine	0.29	0.29	0.19	0.19	0.24	0.24	0.18	0.18	0.20	0.20	0.22	0.22
L-threonine	0.23	0.23	0.07	0.07	0.2	0.2	0.11	0.11	0.17	0.17	0.22	0.22
L-tryptophan	0.04	0.04	-	-	0.02	0.02	-	-	-	-	0.02	0.02
Limestone	0.63	0.63	0.73	0.73	0.60	0.60	0.66	0.66	0.56	0.56	0.60	0.60
Dicalcium phosphate	1.64	1.64	1.26	1.26	1.79	1.79	1.60	1.60	1.65	1.65	1.65	1.65
Common salt	0.20	0.20	0.06	0.06	0.22	0.22	0.13	0.13	0.48	0.48	0.48	0.48
Zinc oxide	-	-	0.30	0.30	-	-	0.30	0.30	-	-	-	-
Choline	0.04	0.04	0.04	0.04	0.04	0.04	0.04	0.04	0.04	0.04	0.04	0.04
Palatant	-	-	0.50	0.50	-	0.00	0.50	0.50	-	-	-	-
Acidifier	-	-	0.60	0.60	-	-	0.60	0.60	-	-	-	-
Mycotoxin adsorbent	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.45	0.45	0.45	0.45
Yeast	-	-	0.25	0.25	-	-	0.25	0.25	-	-	-	-
Tannin	-	0.25	-	0.25	-	0.25	-	0.25	-	0.25	-	0.25
Premix microminerals ^a	0.15	0.15	0.15	0.15	0.15	0.15	0.15	0.15	0.15	0.15	0.15	0.15
Premix vitamins ^b	0.15	0.15	0.15	0.15	0.15	0.15	0.15	0.15	0.15	0.15	0.15	0.15

SD= simple diet; SD+T= simple diet + tannin; CD= complex diet; CD+T= complex diet + tannin; Acidifier composed by organic acids (calcium formate, calcium lactate, citric acid); Added per product kilogram a: selenium, 450 mg; iron, 100 g; copper, 15 g; zinc, 150 g; cobalt, 500 mg; manganese 70g; e iodine 1.4000 mg. Added per product kilogram b: vitamin A, 9.500.000 UI; vitamin D3, 1.400.000 UI; vitamin E, 55.000 mg; vitamin K, 1.900 mg; vitamin B1, 950 mg; vitamin B2, 3.300 mg; vitamin B6, 1.400 mg; vitamin B12, 9.500 mg; pantothenic acid, 9.500 mg; niacin, 14 g; folic acid, 700 mg; and biotin, 38 mg.

total feed intake during a certain period divided by total body weight during this period.

The faecal consistency score (FCS) was measured twice a day (morning and afternoon) during the entire experimental period and classified as “1” (normal faeces), “2” (pasty) and “3” (liquid). Faeces with Scores of 1 and 2 were considered non-diarrhoeal faeces, and those with Score 3 were considered diarrhoeal faeces. The percentage of occurrence of each score was calculated (UTIYAMA et al., 2006) for the periods 0 to 7, 8 to 21 and 22 to 28 days post-weaning and for the whole experimental period.

To measure serum haptoglobin concentration (HPT, mg/dL), blood samples were collected from 24 animals (six per treatment) twice: first at 7 days after weaning and then at 14 days after weaning. This is because the post-weaning period is divided into an acute phase, represented by the first week, and an adaptive phase, in the subsequent days (BURRIN & STOLL, 2003). After the same previous fasting period of 12 hours, 3 mL of blood was collected from the jugular vein (ZIMMERMAN et al., 2012), immediately centrifuged for 10 minutes at 3,000 rpm to obtain the serum fraction and

Table 2 - Calculated nutritional values of post-weaning piglets Pre- starter I, Pre- starter II and Starter diets.

	-----PRE-STARTER I-----				-----PRE-STARTER II-----				-----STARTER-----			
	SD	SD+T	CD	CD+T	SD	SD+T	CD	CD+T	SD	SD+T	CD	CD+T
ME (Mcal/kg)	3.40	3.40	3.40	3.40	3.40	3.40	3.40	3.40	3.35	3.35	3.35	3.35
CP (%)	19.08	19.08	21.72	21.72	18.78	18.78	20.01	20.01	19.15	19.15	18.02	18.02
Crude fiber (%)	1.99	1.99	1.88	1.88	2.09	2.09	1.90	1.90	2.33	2.33	1.89	1.89
Ether extract (%)	1.96	1.96	3.37	3.37	2.08	2.08	2.83	2.83	2.23	2.23	2.33	2.33
Ashes (%)	3.85	3.85	4.27	4.27	3.26	3.26	3.44	3.44	3.03	3.03	3.00	3.00
Calcium (%)	0.85	0.85	0.85	0.85	0.83	0.83	0.83	0.83	0.74	0.74	0.74	0.74
Standardized Phosphorus (%)	0.42	0.42	0.41	0.41	0.41	0.41	0.41	0.41	0.35	0.35	0.36	0.36
Sodium (%)	0.29	0.29	0.28	0.28	0.23	0.23	0.23	0.23	0.21	0.21	0.21	0.21
Lysine ¹ (%)	1.45	1.45	1.47	1.47	1.35	1.35	1.35	1.35	1.28	1.28	1.29	1.29
Methionine ¹ (%)	0.46	0.46	0.43	0.43	0.42	0.42	0.39	0.39	0.45	0.45	0.45	0.45
Methionine+Cystine ¹ (%)	0.78	0.78	0.77	0.77	0.74	0.74	0.74	0.74	0.72	0.72	0.71	0.71
Threonine ¹ (%)	0.83	0.83	0.81	0.81	0.80	0.80	0.79	0.79	0.79	0.79	0.77	0.77
Tryptophan ¹ (%)	0.25	0.25	0.26	0.26	0.26	0.26	0.25	0.25	0.20	0.20	0.20	0.20

SD= simple diet; SD+T= simple diet + tannin; CD= complex diet; CD+T= complex diet + tannin; ME= metabolizable energy; CP= crude protein. ¹Contents expressed as standardized ileal digestible amino acids (calculated according ROSTAGNO et al. (2017)).

stored at -20 °C until laboratorial analysis. The serum haptoglobin concentration (HPT, mg/dL) was determined using the turbidimetric method. This method is generally used for mixed anti-haptoglobin antibodies in samples containing haptoglobin, which form insoluble complexes causing an absorbance change that can be quantified by comparison from a calibrator of a known haptoglobin concentration (RAMAKERS & KREUTZER, 1976).

Statistical Analysis

The data were analyzed by analysis of variance (ANOVA), and the means were compared by the Tukey test at a 5% significance level, after assumption of residual normality and homoscedasticity of variances by Shapiro-Wilk and Bartlett tests. There was no effect of piglets' sex on the analyzed variables, and this factor was therefore excluded from the statistical model. The FCS data were normalized using Box-Cox transformation to attend normality and homoscedasticity prior to ANOVA. For HPT data which failed the presupposition of normality and homoscedasticity, even with Box-Cox transformation, the Kruskal-Wallis non-parametric test was applied. In case of significant differences between treatments ($P < 0.05$), Dunn's multiple comparison test with 5% significance level was used. Differences between treatments were considered significant for $P \leq 0.05$, and differences at the interval of $0.05 < P < 0.10$

were discussed as tendencies. The statistical analyses were performed using the software R (R CORE TEAM, 2022).

RESULTS

Piglet performance

In general, the type of diet (simple or complex) and the tannin inclusion in the diets did not influence piglet performance (Table 3). However, during the Pre-starter II phase (8 to 21 days after weaning), there was a tendency ($P = 0.07$) for piglets to intake 8% more feed in SD (740 g day⁻¹) than in CD (680 g day⁻¹). In the Starter phase (22 to 28 days after weaning), a tendency was observed ($P = 0.06$) for a 6.49% better feed conversion in piglets fed CD.

Faecal consistency score and serum haptoglobin concentration

Diet had an effect on faecal consistency in the first week after weaning, and piglets consuming the CD diet had a lower incidence of liquid ($P < 0.01$) and pasty ($P < 0.01$) faeces compared to those on the SD diet (Table 4). Piglets ingesting diets supplemented with tannin during the Pre-starter I phase (0 to 7 days after weaning) had a lower frequency of liquid ($P = 0.038$) and pasty ($P = 0.013$) faeces. In the Pre-starter II phase (8 to 22 days after weaning), the consumption of diets with tannin extract (SD+T and CD+T) decreased

Table 3 - Performance of weaned piglets submitted to simple and complex diets supplemented or not with tannin.

	-----Treatments-----				-SEM-	-----P-value-----		
	SD	SD+T	CD	CD+T		D	T	D x T
-----Pre-starter I (0-7 days)-----								
IBW, kg	8.06	8.09	7.98	7.95	-	-	-	-
FBW, kg	9.86	9.90	10.04	10.10	0.17	0.17	0.67	0.90
DWG, kg	0.26	0.26	0.29	0.31	0.02	0.29	0.47	0.83
ADFI, kg	0.35	0.36	0.38	0.39	0.02	0.89	0.78	0.42
FCR	1.35	1.39	1.32	1.26	0.20	0.29	0.91	0.54
-----Pre-starter II (8-21 days)-----								
IBW, kg	9.86	9.90	10.04	10.10	0.17	0.17	0.67	0.90
FBW, kg	16.57	16.99	17.06	17.42	0.49	0.82	0.48	0.74
DWG, kg	0.48	0.50	0.50	0.52	0.02	0.79	0.63	0.84
ADFI, kg	0.74	0.69	0.68	0.71	0.03	0.07	0.98	0.50
FCR	1.55	1.38	1.37	1.37	0.08	0.11	0.16	0.14
-----Starter (22-28 days)-----								
IBW, kg	16.57	16.99	17.06	17.42	0.49	0.82	0.48	0.74
FBW, kg	20.96	21.59	21.65	22.00	0.64	0.76	0.71	0.73
DWG, kg	0.64	0.65	0.65	0.64	0.03	0.90	0.88	0.73
ADFI, kg	1.15	1.11	1.09	1.03	0.04	0.18	0.92	0.79
FCR	1.79	1.71	1.67	1.61	0.09	0.06	0.24	0.90
-----Whole period (0-28 days)-----								
IBW, kg	8.06	8.09	7.98	7.95	-	-	-	-
FBW, kg	20.96	21.59	21.65	22.00	0.64	0.76	0.71	0.73
DWG, kg	0.48	0.48	0.48	0.49	0.02	0.76	0.71	0.73
ADFI, kg	0.77	0.73	0.71	0.71	0.02	0.19	0.87	0.38
FCR	1.60	1.52	1.48	1.48	0.09	0.17	0.46	0.49

SD= simple diet; SD+T= simple diet + tannin; CD=complex diet; CD+T= complex diet + tannin; D=diet; T=tannin; IBW = initial body weight; FBW= final body weight; DWG= daily weight gain; ADFI= average daily feed intake; FCR= feed conversion ratio; D x T= interaction diet x tannin.

the proportion of piglets with pasty faeces ($P = 0.018$) and had a tendency to reduce liquid faeces ($P = 0.082$).

In the serum haptoglobin concentrations (HPT) from two blood collections on 7 and 14 days after weaning (Table 5), the inclusion of tannin in diets (SD+T and CD+T) reduced the serum concentration of HPT ($P = 0.029$) compared to SD and CD diets. There was a tendency of interaction between the factors of diet type (simple and complex) and inclusion of tannin ($P = 0.063$), where the HPT levels were lower in piglets fed diets supplemented with tannin.

DISCUSSION

Traditionally, tannins are known as anti-nutritional compounds with negative effects on diet digestibility, especially protein digestibility, and pig

performance (MYRIE et al., 2008; REDONDO et al., 2014). In addition, tannins can affect feed palatability due to their bitter taste and astringency when fed at high concentrations (BRUINS et al., 2011; STEINER & SYED, 2015).

An absence of effects of tannin inclusion in piglet feed on performance, regardless of the diet type, was found by other authors (MYRIE et al., 2008). However, the inclusion of tannins in the diet increased feed efficiency in piglets (BIAGI et al., 2010). BIAGI et al. (2010) used 4.5 g kg^{-1} of chestnut tannin extract in the diet, which is 1.8 times higher than the dose used in our experiment ($1,850 \text{ mg kg}^{-1}$). An improvement in weaned piglet performance with a diet containing quebracho and chestnut tannin extract (0.75%) was also reported by CAPRARULO et al. (2020). Differences in the source and levels of tannin inclusion, as well as

Table 4 - Frequencies of daily faecal consistency score from weaned piglets submitted to simple and complex diets supplemented or not with tannin.

	-----Treatments-----				---SEM---	-----P-value-----		
	SD	SD+T	CD	CD+T		Diet	Tannin	D x T
-----Pre-starter I (0-7 days)-----								
S1(%)	85.39	91.23	94.81	99.68	1.272	0.002	0.078	0.061
S2(%)	7.14	5.19	3.89	0.32	0.772	0.001	0.013	0.641
S3(%)	7.47	3.58	1.30	0.00	0.700	0.001	0.038	0.112
-----Pre-starter II (8-21 days)-----								
S1(%)	90.58	95.29	94.48	97.73	0.943	0.470	0.059	0.979
S2(%)	5.68	2.40	4.52	1.30	0.671	0.537	0.018	0.865
S3(%)	3.74	2.31	1.00	0.97	0.871	0.787	0.082	0.352
-----Starter (22-28 days)-----								
S1(%)	91.56	96.43	85.71	85.71	10.60	0.005	0.012	0.566
S2(%)	5.84	2.27	4.55	4.55	139.55	0.265	0.511	0.323
S3(%)	2.59	1.30	9.74	9.74	152.42	0.010	0.002	0.023
-----Whole period (0-28 days)-----								
S1(%)	89.53	94.56	92.37	92.37	7.58	0.237	0.012	0.903
S2(%)	6.09	3.57	4.39	4.38	115.67	0.243	0.012	0.981
S3(%)	4.38	1.87	3.24	3.24	129.16	0.340	0.062	0.516

SD= simple diet; SD+T= simple diet + tannin; CD= complex diet; CD+T= complex diet +tannin; S1= normal faeces; S2= pasty faeces; S3= liquid faeces; SEM=Standard Error of the Mean; D x T= interaction diet x tannin.

the sanitary challenges for the animals, among studies may explain the different results.

In intensive swine farming, the supply of complex diets, with high digestibility and high nutritional levels, for piglets is consolidated (DOUGLAS et al., 2014). It is possible that our results are associated with the chemical and physical characteristics of the experimental diets. The simple diets, in the Pre-starter I and Pre-starter II phases, had lower concentrations of digestible ingredients and dairy products, in addition to a higher concentration of non-starch polysaccharides (NSP) with barley inclusion, which might have presented a nutritional challenge for piglets and reduced performance. However, the experimental diets were isonutritive, which may have minimized the differences between the simple and complex diets. In addition, the simple diets contained 14% barley, a cereal with high beta-glucan levels (WOOD, 2004), generally ranging from 2.68% to 4.74% (NISHANTHA et al., 2018). Beta-glucans are polysaccharides that can improve intestinal development and health in weaned piglets (VOLMAN et al., 2008).

Diarrhoea is defined by an increased defecation frequency and by faeces with a high

concentration of water and a low dry matter content (JONES et al., 2019). Post-weaning diarrhoea is a major problem because it increases morbidity and mortality and affects piglet health and performance (CAMPBELL et al., 2013). The reduced incidence of diarrhoea in animals fed complex diets supplemented with tannin may be associated with these polyphenols' capacity to decrease intestinal permeability through an increase in the cellular contents of occludin, claudin and zonula occludens proteins of tight junctions, as well as an inhibition of myosin light-chain kinase activation (DE SANTIS et al., 2015). Tannins have great potential to prevent microbial infections and reduce coliforms associated with post-weaning diarrhoea, promoting animal health (DELL'ANNO et al., 2021). However, the underlying mechanisms need to be better understood (GIRARD & BEE, 2020).

One of the host's reactions to microbial infections is by APR (ECKERSALL, 2000). The APR changes the concentrations of some proteins, including an increase in HPT concentration. In pigs, HPT is one of the main proteins from APR and synthesised by the liver (HULTÉN et al., 2003). A lower concentration of HPT in tannin-supplemented

Table 5 - Serum concentration of haptoglobin (HPT, mg/dL) in weaned piglets fed simple or complex diets supplemented or not with tannin.

	-----Treatments-----				--SEM--	-----P value-----		
	SD	SD+T	CD	CD+T		Diet	Tannin	D x T
HPT (mg/dL)	25.25	18.28	23.18	22.60	1.65	0.502	0.029	0.063

SD= simple diet; SD+T= simple diet + tannin; CD= complex diet; CD+T= complex diet + tannin; SEM=Standard error of mean; HPT= serum concentration of haptoglobin.

simple diets indicates a reduction in inflammatory processes, which has also been found in ruminants (RODRIGUES et al., 2019). According to previous findings, HPT is a biomarker for inflammatory processes (ECKERSALL, 2000), and phytogetic ingredients help regulate inflammatory and acute-phase responses (YANG et al., 2010). Several *in vitro* studies have indicated that tannins can reduce pathogenic bacterial development, showing the efficiency of tannins as prebiotics. In the case of enterotoxigenic *Escherichia coli*, the main action mechanisms attributed to tannins are the reduction in the fimbriae's ability to adhere to the intestinal epithelium and the impediment of biofilm formation and enterotoxin action (GIRARD & BEE, 2020).

CONCLUSIONS

The inclusion of 1,850 mg kg⁻¹ of black wattle (*Acacia mearnsii*) condensed tannin extract in piglet diets had no effect on piglet performance, even when a simple diet with less digestible ingredients was fed. Piglet fed diets with tannin supplementation showed a lower incidence of diarrhoea in the first week after weaning and a reduction in haptoglobin, indicating an improvement in gut health and a decrease in inflammatory responses.

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DECLARATION OF CONFLICT OF INTEREST

The authors declare no conflict of interest. The founding sponsors had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript, and in the decision to publish the results.

AUTHORS' CONTRIBUTIONS

All authors contributed equally for the conception and writing of the manuscript. All authors critically revised the manuscript and approved of the final version.

BIOETHICS AND BIOSSECURITY COMMITTEE APPROVAL

The procedures adopted in this experiment followed the provisions of Federal Law N° 11,794 of 8 October 2008 and Decree N° 6,899 of 15 July 2009, under Case N° 1888290720 of the local Ethics Committee of Animal Use (CEUA).

REFERENCES

- BARBEHENN, R. V.; CONSTABEL, P. C. Tannins in plant-herbivore interactions. **Phytochemistry**, v.72, n.13, p.1551–1565, 2011. Available from: <<https://doi.org/10.1016/j.phytochem.2011.01.040>>. Accessed: Aug. 28, 2022. doi: 10.1016/J.PHYTOCHEM.2011.01.040.
- BIAGI, G. et al. Effect of tannins on growth performance and intestinal ecosystem in weaned piglets. **Archives of Animal Nutrition**, v. 64, n.2, p.121–135, 2010. Available from: <<https://doi.org/10.1080/17450390903461584>>. Accessed: Aug. 28, 2022. doi: 10.1080/17450390903461584.
- BRUINS, M. J. et al. Black tea reduces diarrhoea prevalence but decreases growth performance in enterotoxigenic *Escherichia coli*-infected post-weaning piglets. **Journal of Animal Physiology and Animal Nutrition**, v.95, n.3, p.388–398, 2011. Available from: <<https://doi.org/10.1111/J.1439-0396.2010.01066.X>>. Accessed: Aug. 28, 2022. doi: 10.1111/J.1439-0396.2010.01066.X.
- BRUS, M. et al. Effect of hydrolysable tannins on proliferation of small intestinal porcine and human enterocytes. **8th International Symposium on the Mediterranean Pig**, October 10th –12th, 2013. p.131–134, 2013. Available from: <<http://aas.bf.uni-lj.si/zootehnika/supl/4-2013/PDF/4-2013-131-134.pdf>>. Accessed: Aug. 28, 2022.
- BURRIN, D.; STOLL, B. Intestinal nutrient requirements in weaning pigs. In: **Weaning the pig: Concepts and Consequences**. Edited by Pluske JR, Verstegen MWA, Le Dividich J. The Netherlands: Wageningen Academic Publishers, p.301-305, 2003. Available from: <<https://doi.org/10.3920/978-90-8686-513-0>>. Accessed: Aug. 28, 2022.

- CAMPBELL, J. M. et al. The biological stress of early weaned piglets. **Journal of animal science and biotechnology**, v.4, n.1, p.1-4, 2013. Available from: <<https://doi.org/10.1186/2049-1891-4-19>>. Accessed: Jan. 16, 2023. doi: 10.1186/2049-1891-4-19.
- CAPRARULO, V. et al. Evaluation of dietary administration of chestnut and quebracho tannins on growth, serum metabolites and fecal parameters of weaned piglets. **Animals**, v.10, p.1945, 2020. Available from: <<https://doi.org/10.3390/ANI10111945>>. Accessed: Aug. 28, 2022. doi: 10.3390/ANI10111945.
- DE SANTIS, S. et al. Nutritional keys for intestinal barrier modulation. **Frontiers in immunology**, v.6, p.612, 2015. Available from: <<https://doi.org/10.3389/fimmu.2015.00612>>. Accessed: Jan. 16, 2023. doi: 10.3389/fimmu.2015.00612.
- DELL'ANNO, M. et al. Evaluation of tannin extracts, leonardite and tributyrin supplementation on diarrhoea incidence and gut microbiota of weaned piglets. **Animals**, v.11, p.1693, 2021. Available from: <<https://doi.org/10.3390/ani11061693>>. Accessed: Aug. 28, 2022. doi: 10.3390/ani11061693.
- DOUGLAS, S. L. et al. High specification starter diets improve the performance of low birth weight pigs to 10 weeks of age. **Journal of Animal Science**, v.92, n.10, p.4741-4750, 2014. Available from: <<https://doi.org/10.2527/JAS.2014-7625>>. Accessed: Aug. 28, 2022. doi: 10.2527/JAS.2014-7625.
- ECKERSALL, P. D. Recent advances and future prospects for the use of acute phase proteins as markers of disease in animals. **Revue de Medecine Veterinaire**, v.151, n.7, p.577-584, 2000. Available from: <<https://citeseerx.ist.psu.edu/document?repid=rep1&type=pdf&doi=4b95353eae0966a8406794fd9df639c012855dd9>>. Accessed: Jan. 16, 2023.
- FIESEL, A. et al. Effects of dietary polyphenol-rich plant products from grape or hop on pro-inflammatory gene expression in the intestine, nutrient digestibility and faecal microbiota of weaned pigs. **BMC veterinary research**, v.10, n.1, p.1-11, 2014. Available from: <<https://doi.org/10.1186/s12917-014-0196-5>>. Accessed: Jan. 16, 2023. doi: 10.1186/s12917-014-0196-5.
- GIRARD, M.; BEE, G. Invited review: Tannins as a potential alternative to antibiotics to prevent coliform diarrhea in weaned pigs. **Animal**, v.14, n.1, p.95-107, 2020. Available from: <<https://doi.org/10.1017/S1751731119002143>>. Accessed: Aug. 28, 2022. doi: 10.1017/S1751731119002143.
- HULTÉN, C. et al. Interleukin 6, serum amyloid A and haptoglobin as markers of treatment efficacy in pigs experimentally infected with *Actinobacillus pleuropneumoniae*. **Veterinary Microbiology**, v.95, n.1-2, p.75-89, 2003. Available from: <[https://doi.org/10.1016/S0378-1135\(03\)00136-6](https://doi.org/10.1016/S0378-1135(03)00136-6)>. Accessed: Aug. 28, 2022. doi: 10.1016/S0378-1135(03)00136-6.
- HUSSAIN, T. et al. The role of polyphenols in regulation of heat shock proteins and gut microbiota in weaning stress. **Oxidative Medicine and Cellular Longevity**, v.2021, ID 6676444, 2021. Available from: <<https://doi.org/10.1155/2021/6676444>>. Accessed: Jan. 16, 2023. doi: 10.1155/2021/6676444.
- JONES, S.L. et al. Diseases of the alimentary tract. In: **Large animal internal medicine**. Elsevier, p.702-920, 2019. Available from: <<https://doi.org/10.1016/B978-0-323-55445-9.00032-X>>. Accessed: Aug. 28, 2022. doi: 10.1016/B978-0-323-55445-9.00032-X.
- LAU, D. W. et al. An assay to estimate tannins added to postmortem turkey meat. **Journal of Agricultural and Food Chemistry**, v.51, n.23, p.6640-6644, 2003. Available from: <<https://doi.org/10.1021/JF030121T>>. Accessed: Aug. 28, 2022. doi: 10.1021/JF030121T.
- LIU, H. et al. Effects of hydrolysable tannins as zinc oxide substitutes on antioxidant status, immune function, intestinal morphology, and digestive enzyme activities in weaned piglets. **Animals**, v.10, n.5, p.757, 2020. Available from: <<https://doi.org/10.3390/ANI10050757>>. Accessed: Aug. 28, 2022. doi: 10.3390/ANI10050757.
- MAHFUZ, S. et al. Phenolic compounds as natural feed additives in poultry and swine diets: a review. **Journal of Animal Science and Biotechnology**, v.12, n.1, p.1-18, 2021. Available from: <<https://doi.org/10.1186/S40104-021-00565-3>>. Accessed: Aug. 28, 2022. doi: 10.1186/S40104-021-00565-3.
- MARSHALL, B. M.; LEVY, S. B. Food animals and antimicrobials: impacts on human health. **Clinical Microbiology Reviews**, v.24, n.4, p.718-733, 2011. Available from: <<https://doi.org/10.1128/CMR.00002-11>>. Accessed: Jan. 16, 2023. doi: 10.1128/CMR.00002-11.
- MARISCAL-LANDÍN, G. et al. Effect of tannins in sorghum on amino acid ileal digestibility and on trypsin (E.C.2.4.21.4) and chymotrypsin (E.C.2.4.21.1) activity of growing pigs. **Animal Feed Science and Technology**, v.117, n.3-4, p.245-264, 2004. Available from: <<https://doi.org/10.1016/J.ANIFEEDSCI.2004.09.001>>. Accessed: Aug. 28, 2022. doi: 10.1016/J.ANIFEEDSCI.2004.09.001.
- MUELLER-HARVEY, I. Unravelling the conundrum of tannins in animal nutrition and health. **Journal of the Science of Food and Agriculture**, v.86, n.13, p.2010-2037, 2006. Available from: <<https://doi.org/10.1002/jsfa.2577>>. Accessed: Jan. 16, 2023. doi: 10.1002/jsfa.2577.
- MYRIE, S. B. et al. Effect of common antinutritive factors and fibrous feedstuffs in pig diets on amino acid digestibilities with special emphasis on threonine. **Journal of Animal Science**, v.86, n.3, p.609-619, 2008. Available from: <<https://doi.org/10.2527/JAS.2006-79>>. Accessed: Aug. 28, 2022. doi: 10.2527/JAS.2006-79.
- NISHANTHA, M. D. L. C. et al. Direct comparison of β -glucan content in wild and cultivated barley. **International Journal of Food Properties**, v.21, n.1, p.2218-2228, 2018. Available from: <<https://doi.org/10.1080/10942912.2018.1500486>>. Accessed: Jan. 16, 2023. doi: 10.1080/10942912.2018.1500486.
- OLIVEIRA, V. et al. FORMULAE feed formulation software®: a tool for diets formulation. **Revista Brasileira de Engenharia de Biosistemas**, v.13, n.4, p.349-354, 2019. Available from: <<https://doi.org/10.18011/bioeng2019v13n4p349-354>>. Accessed: Aug. 28, 2022. doi: 10.18011/bioeng2019v13n4p349-354.
- PALOMBO, E. A. Phytochemicals from traditional medicinal plants used in the treatment of diarrhoea: modes of action and effects on intestinal function. **Phytotherapy Research**, v.20, n.9, p.717-724, 2006. Available from: <<https://doi.org/10.1002/PTR.1907>>. Accessed: Aug. 28, 2022. doi: 10.1002/PTR.1907.
- PLUSKE, J. R. et al. Maintenance of villus height and crypt depth, and enhancement of disaccharide digestion and monosaccharide absorption, in piglets fed on cows' whole milk after weaning. **British Journal of Nutrition**, v.76, n.3, p.409-422, 1996. Available from: <<https://doi.org/10.1079/BJN19960046>>. Accessed: Jan. 16, 2023. doi: 10.1079/BJN19960046.

- R CORE TEAM. **R**: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria, 2022. Available from: <<https://www.R-project.org>>. Accessed: Aug. 28, 2022.
- RAMAKERS, J. M.; KREUTZER, H. J. H. Turbidimetric Determination of Haptoglobin. **Clinical Chemistry and Laboratory Medicine**, v.14, n.1–12, p.407–410, 1976. Available from: <<https://doi.org/10.1515/cclm.1976.14.1-12.407>>. Accessed: Aug. 28, 2022. doi: 10.1515/cclm.1976.14.1-12.407.
- REDONDO, L. M. et al. Perspectives in the use of tannins as alternative to antimicrobial growth promoter factors in poultry. **Frontiers in Microbiology**, v.5, n.MAR, p.118, 2014. Available from: <<https://doi.org/10.3389/FMICB.2014.00118/XML/NLM>>. Accessed: Aug. 28, 2022. doi: 10.3389/FMICB.2014.00118/XML/NLM.
- RODRIGUES, R. O. et al. Productive and physiological responses of lactating dairy cows supplemented with phytochemical ingredients. **Translational Animal Science**, v.3, n.4, p.1133–1142, 2019. Available from: <<https://doi.org/10.1093/tas/txz108>>. Accessed: Jan. 16, 2023. doi: 10.1093/tas/txz108.
- REGGI, S. et al. In vitro digestion of chestnut and quebracho tannin extracts: Antimicrobial effect, antioxidant capacity and cytomodulatory activity in swine intestinal IPEC-J2 cells. **Animals**, v.10, n.2, p.195, 2020. Available from: <<https://doi.org/10.3390/ani10020195>>. Accessed: Jan. 16, 2023. doi: 10.3390/ani10020195.
- ROSTAGNO, H. S. et al. **Tabelas brasileiras para aves e suínos**. Viçosa: UFV, 403p, 2017.
- STEINER, T.; SYED, B. Phytochemical Feed Additives in Animal Nutrition. **Medicinal and Aromatic Plants of the World**, 403–423, 2015. Available from: <https://doi.org/10.1007/978-94-017-9810-5_20>. Accessed: Jan. 16, 2023. doi:10.1007/978-94-017-9810-5_20.
- UTIYAMA, C. E. et al. Effects of antimicrobials, prebiotics, probiotics and herbal extracts on intestinal microbiology, diarrhea incidence and performance of weaning pigs. **Revista Brasileira de Zootecnia**, 35, 2359–2367, 2006. Available from: <<https://doi.org/10.1590/S1516-35982006000800023>>. Accessed: Jan. 16, 2023. doi: 10.1590/S1516-35982006000800023.
- VOLMAN, J. J. et al. Dietary modulation of immune function by β -glucans. **Physiology & Behavior**, v.94, n.2, p.276–284, 2008. Available from: <<https://doi.org/10.1016/J.PHYSBEH.2007.11.045>>. Accessed: Aug. 28, 2022. doi: 10.1016/J.PHYSBEH.2007.11.045.
- WOOD, P. J. Relationships between solution properties of cereal β -glucans and physiological effects: a review. **Trends in Food Science & Technology**, v.15, n.6, p.313–320, 2004. Available from: <<https://doi.org/10.1016/J.TIFS.2003.03.001>>. Accessed: Aug. 28, 2022. doi: 10.1016/J.TIFS.2003.03.001.
- YANG, C. et al. Phytochemical compounds as alternatives to in-feed antibiotics: potentials and challenges in application. **Pathogens**, v.4, n.1, p.137–156, 2015. Available from: <<https://doi.org/10.3390/pathogens4010137>>. Accessed: Jan. 16, 2023. doi: 10.3390/pathogens4010137.
- ZIMMERMAN, J. J. et al. **Diseases of swine**. John Wiley & Sons, 2012.