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APPLICATION OF SUNFLOWER MEAL IN THE FEEDING OF BROILER CHICKENS–POTENTIALS AND CONSTRAINTS

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1. BACKGROUND OF THE WORK AND ITS AIMS

Food demand and food value chain globalisation are increasing very fast; at the same time, numerous environmental factors are continuously influencing crop productivity and stability. This necessitates the practice of a sustainable poultry production system through the use of locally available, cheaper low-protein diets (El-Deek et al., 2020). Globally, poultry meat consumption is projected to grow by about 21% and reach 173 Mt of ready-to-cook meat, which accounts for 62% of additional meat consumption. This will provide 45% of the protein consumed from all meat sources by 2034 (OECD/FAO, 2025). Similarly, world import demand for poultry meat is expected to increase by 2.5 million tonnes by 2035 (EC, 2025).

In the case of broiler chicks, feed cost accounts for about 70% of the total production cost. A recent study reported that, over a four-year production period, the average feed cost accounts for 71.9% (69.6 to 76.1%) of the total production cost (Adaszyńska-Skwirzyńska et al., 2025). Feed cost and the price of both egg and meat increase in parallel; therefore, the higher the feed cost, the more expensive and less affordable poultry products become for most people worldwide. Protein is the most expensive feed component. The preferred source of protein is soybean meal.

Soybean production expansion has caused deforestation, biodiversity loss, and food security challenges (Peng et al., 2025) and trade dynamics are driven by climate change. Similarly, the global soybean production deficit in 2012 was attributable to climate change (Hamed et al., 2025). Therefore, climate change and soybean importation are the root cause for the continuous price increment in poultry feed, insecurity, and high carbon footprint (Dotas et al., 2025) as a result, feed manufacturing industries, poultry farmers, and researchers are looking for locally available, cheaper alternative protein sources. Therefore, the use of industrial by-products and locally grown protein crops as poultry feeds are expected to increase in the future. Possible alternatives are legume seeds, extracted meals, dried distilled grains with solubles, meat meals, fish meal, insect meal, and seaweeds. The challenge with such feed ingredients is that there is no clearly determined information regarding their maximum inclusion rates (Dotas et al., 2025). Such challenges are evident in optimising sunflower meal (SM) inclusion, mitigating the knowledge gap and inconsistencies in including SM in broiler diets. Therefore, to ensure sustainable broiler production and reduce the environmental impact of meat production, the use of locally available cheaper protein diets and reducing soybean importation is timely needed. This can be

mitigated by identifying and incorporating locally available alternatives in broiler feed formulation, a subject of growing interest to reduce dependency on traditional sources and improve sustainability. In this case SM could be one of the potential alternatives.

Cell wall of SM contains non-starch polysaccharides (NSPs), which are known to increase digesta viscosity, reduce nutrient utilization, and consequently depress growth in chickens (Senkoylu and Dale, 1999). This can be mitigated through the use of exogenous enzyme supplementation. The use of exogenous enzymes improves the value of agro-industrial and agroforestry wastes used as animal feed by enhancing nutrient availability, digestibility, and reducing anti-nutritional factors. Although animals produce their own digestive enzymes, they cannot fully break down these materials, so enzyme treatment has become an effective strategy to boost productivity and animal performance (Ojha et al., 2019; Ravindran, 2013).

The above-mentioned characteristics of SM make it a better alternative to minimise the dependency on the traditional crops in chicken production, make broiler production sustainable, and to reduce production cost and the environmental impact of importing soybean meal far from Latin America. Though limited information is available on the maximum inclusion levels of SM in broiler diets, Such et al. (2024) reported that SM can be used up to 30% in the diets of pullets and laying hens. They also concluded SM could entirely replace soybean meal. If pullets can tolerate SM inclusion up to 30%, then there is a potential to include SM in the broiler diet as well. As a result, the objectives of the current study were as follows:

- To investigate and compare the inclusion of both HFSM and LFSM at 20% and 30% levels in the broiler grower and broiler finisher diets and their impact on production performance, carcass traits, nutrient digestibility, nitrogen excretion, N retention, excreta N contents, caecal short-chain fatty acid contents, and digesta viscosity.
- To evaluate the amino acid (AA) digestibility of broiler diets containing HFSM and LFSM in the whole ileum during the grower phase, and the proximal jejunum, proximal ileum and distal ileum during the finisher phase.
- To compare the AA absorption between the jejunal and ileal parts of the small intestine during the finisher phase in order to get more understandings on how SM can affect the dynamics of AA absorption in the different parts of the small intestine.
- To investigate feeding sunflower meal and extra phytase effects on the production traits, nutrient digestibility, organ weight, gut content pH, histomorphology, intestinal length, and short chain fatty acid contents.

2. MATERIALS AND METHODS

In this PhD work two experiments were conducted to study the nutritional effects of SM fed in the diets of broiler chickens.

2.1. Materials and methods applied in both experiments

2.1.1. Birds and housing

A floor pen trial was carried out at the experimental farm of the Department of Animal Nutrition and Nutrition Physiology, Georgikon Campus, Hungarian University of Agriculture and Life Sciences, under the licence number MAB-1/2023 after approval by the campus ethics committee. In experiment 1, a total of 600 Ross 308 male birds were purchased from a local hatchery (Gallus Ltd., Devecser, Hungary) and randomly allocated to five treatments, each replicated five times containing 24 birds within an environmentally computer-controlled house. While in experiment 2, A total of 576-day-old male Ross 308 chickens were purchased from the same commercial hatchery Gallus Ltd., and randomly allocated to 24 pens. Four dietary treatments were applied, with 144 chickens per treatment and six replicates of 24 birds each. The lighting programme was set according to the guidelines given by Aviagen for Ross 308 (Aviagen, 2018). Each bird was vaccinated against infectious bronchitis, Newcastle disease, and infectious bursal disease in the hatchery.

2.1.2. Chemical analysis

The proximate nutrients of the experimental diets were analysed with well-known standard methods. The neutral detergent fibre (NDF) was determined sequentially as described by VanSoest et al. (1991) and expressed on Ash free basis. The gross energy (GE) of the diets and excreta samples was analysed using a bomb calorimeter (IKA C6000, IKA-Werke GmbH & Co., Breisgau, Germany). The starch content was measured by the polarimetric method according to European Directive 152/2009 (EC, 2025). The amino acid content of the feeds and digesta samples was analysed using an automatic amino acid analyser (Ingos Amino Acid Analyzer AAA 400, INGOS s.r.o., Prague, Czech Republic) according to the ISO 13903:2005 standard(ISO 13903, 2005). The titanium dioxide (TiO_2) determination was carried out by an UV spectrophotometer (Jenway 6100, Bibby Scientific Limited, Staffordshire, UK), with absorbance measurements taken at a wavelength of 410 nm, according to Short et al. (1996).

2.1.3. Short-chain fatty acid (SCFA) determination

After euthanizing the birds, cecum chyme samples were collected for SCFA analysis and immediately stored at −20 °C until analysis. Samples were thawed and prepared for gas chromatographic SCFA determination according to Atteh et al. (2008). Frozen samples were mixed and thawed thoroughly. Then, 250 µL of the digesta was mixed with 600 µL of 1.11 M HCl. The gas chromatograph, equipped with a 30 m (0.25 mm 178 i.d.) fused silica Nukol column (Supelco Inc., Bellefonte, PA, USA), used a flame ionisation detector with a 1:50 split injector. The injector volume was 1 µL at 220 °C, and detection occurred at 250 °C. Helium was used as a carrier gas at a pressure of 83 kPa. Calibration was performed using standard SCFA mixtures (1, 4, 8, and 20 mM) of acetate, propionate, n-butyrate, and n-valerate.

2.2. Materials and methods of the first experiment

2.2.1. Experimental diets

Feed was formulated according to the guidelines of the breeder company (Aviagen, 2018). Five isocaloric and isonitrogenous diets were prepared. The five treatments consisted of a soybean-wheat-corn based control diet (C), and diets that contained HFSM and LFSM at inclusion levels of 20% (HFSM20, LFSM20) and 30% (HFSM30, LFSM30); these were fed in the grower (days 11–24) and finisher (days 25–38) phases. Diets in the starter phase (day 0–10) did not contain SM. The composition of the diets is shown in Table. 1. The measured nutrient content of the diets was close to the predicted ones (Table 2).

Table 1. Composition of the experimental diets (g/kg)

Ingredients	Starter	Grower					Finisher				
		C	HFS M20	HFS M30	LFS M20	LFS M30	C	HFS M20	HFS M30	LFS M20	LFS M30
Corn	392	403	324	284	401	402	469	386	347	465	459
Wheat	100	100	100	100	100	100	100	100	100	100	100
Extracted Soybean	400	376	221	143	161	53	317	163	85	103	0
HFSM [1]	55	0	200	300	0	0	0	200	300	0	0
LFSM [2]	0	0	0	0	200	300	0	0	0	200	300
Sunflower oil	0	75	108	124	89	95	71	105	121	85	93
MCP [3]	16	15	15	16	16	16	13	14	14	14	14
Limestone	18	15	13	13	14	13	13	12	11	12	11
Premix [4]	5	5	5	5	5	5	5	5	5	5	5
Salt	3	3	3	3	3	3	3	3	3	3	3
NaHCO3	1	1	1	1	1	1	1	1	1	1	1
Biolys	4	2	5	7	6	8	3	6	8	7	9
Methionine	4	3	3	2	2	2	3	3	2	2	2
Threonine	1	1	1	1	1	1	1	1	2	2	2
Valine	1.0	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
Total (g)	1000	1000	1000	1000	1000	1000	1000	1000	1000	1000	1000

C—control diet, [1] High-fibre sunflower meal, [2] Low-fibre sunflower meal, HFSM20—diet that contains 20% HFSM, HFSM30—diet that contains 30% HFSM, LFSM20—diet that contains 20% LFSM, LFSM30—diet that contains 30% LFSM, [3] Monocalcium phosphate; [4] Premix was supplied by Agrofeed Ltd. (Győr, Hungary). The active ingredients contained in the premix were as follows (NE per kg of diet): vitamin A—2,000,000 NE, vitamin D3—600,000 NE, vitamin E—6000 mg, menadione—400 mg, thiamine—436 mg, riboflavin—1200 mg, pyridoxin HCl—600 mg, cyanocobalamin—4 mg, niacin—6254 mg, pantothenic acid—1825 mg, folic acid—300 mg, biotin—30 mg, betaine—30,000 mg, BHT—79.5 mg, BHA—79.5 mg, citric acid—71.5 mg, Zn (as ZnO)—8000 mg, Zn (as 3b607)—8000 mg, Cu (as 3b413)—2000 mg, Fe (as $FeSO_4H_2O$)—10,000 mg, Mn (as MnO)—10,000 mg, Mn (as 3b506)—10,000 mg, I [as $Ca(IO_3)2$]—300 mg, Se (as $C_5H_{11}NO_2Se$)—40 mg, endo-1.4-beta-xylanase—244,000 U, Endo-1.3(4)-beta-glucanase—30,400 U, 6-phytase—100,000 FTU.

Table 2. Measured nutrient content of the experimental diets (g/kg).

Nutrients	Starter	Growers					Finishers				
		C	HFSM 20	HFSM 30	LFSM 20	LFSM 30	C	HFSM 20	HFSM 30	LFSM 20	LFSM 30
Dry matter	896.2	896.3	905.8	905.5	896.5	913.1	894.2	898.1	900.1	899.3	897.5
Crude protein	232.8	220.9	219.5	219.5	220.9	218.7	209.4	200.4	194.1	200.9	199.9
Crude fat	73.7	90.5	129.4	134.8	114.3	115.0	90.7	102.3	131.0	98.3	103.3
Crude fibre	26.8	34.0	60.2	76.7	55.3	65.7	35.0	71.6	83.9	46.1	55.7
Ash	64.8	64.1	62.9	64.4	65.5	65.3	62.5	62.0	61.3	62.3	63.4
Calcium	10.6	10.2	10.0	10.4	9.9	10.3	9.2	9.3	9.5	9.1	9.6
Phosphorus	6.1	6.6	7.8	8.5	7.3	9.0	6.2	7.8	8.2	7.7	8.3
Starch	321.6	361.4	302.2	277.0	326.8	309.9	381.6	329.0	309.8	376.1	362.2
GE [1] (kJ/g)	176.1	180.8	188.3	192.2	188.6	193.9	178.6	188.1	189.8	185.3	185.7
NDF [2]	149.9	135.6	155.8	163.2	155.7	166.3	137.9	156.6	165.0	159.1	168.0
Arginine	15.5	14.6	15.2	15.4	15.2	15.4	13.5	13.0	12.7	14.5	12.8
Isoleucine	9.8	9.2	09.1	08.8	8.8	8.3	8.6	7.9	7.4	7.5	7.3
Lysine	14.9	12.5	12.8	12.8	12.7	12.6	11.7	11.3	11.2	11.1	10.8
Methionine	6.5	6.0	6.0	5.9	6.0	6.7	5.6	5.7	5.5	5.8	5.7
Threonine	9.7	9.2	9.3	9.0	9.1	8.9	8.7	8.2	9.0	9.1	9.2
Valine	3.6	3.4	3.5	3.5	3.5	3.5	3.3	3.2	3.2	3.2	3.3

C—control diet, HFSM—High-fibre sunflower meal, LFSM—Low-fibre sunflower meal, HFSM20—diet that contains 20% HFSM, HFSM30—diet that contains 30% HFSM, LFSM20—diet that contains 20% LFSM, LFSM30—diet that contains 30% LFSM, [1] Gross energy, [2] Neutral detergent fibre.

2.2.2. Samplings and measurements

2.2.2.1. Production traits and digestibility trial

Production parameters such as the FI and body weight (BW) of all chickens were measured at the end of the grower (day 24) and finisher (day 38) phases on pen basis. An indigestible marker, TiO_2, was mixed at a rate of 5 g/kg in the grower and finisher diets. On day 24, 16 chickens from each treatment group were selected randomly and placed into 40 balance cages in pairs, representing eight replicates of each treatment. Again, on day 38 a total of 40 chickens, 8 birds per treatment, were also assigned to balanced cages for carrying out the digestibility trial. After 2 days of adaptation (on days 26 and 27, and days 40 and 41) representative excreta samples were collected.

The daily samples were homogenised, and around 50 g of excreta were frozen and stored until further analysis. On days 27 and 41, all the animals of the digestibility trial were euthanised and slaughtered in compliance with the animal welfare legislation (Hungarian Government Decree 40/2013). The abdominal cavity of

the animals was opened immediately and the digesta of the whole jejunum for viscosity, the caecal content of the left sacks for SCFA analysis, and the whole ileum for AA digestibility was collected. The gut contents were gently squeezed and poured into Eppendorf tubes (0.5 mL) and stored in a refrigerator at −20 °C prior to AA, viscosity and SCFA analyses.

At the end of the grower phase, the gut contents of two chickens from the same cage were pooled. On day 41 digesta from the distal jejunum, proximal ileum and distal ileum was collected for AA digestibility. Each intestinal part was cut into short pieces, and the digesta was gently squeezed out, homogenized and stored in Eppendorf tubes at −20 °C until analysis. After the collection of gut contents, the carcass composition of chickens was also determined. The following parameters were measured: carcass weight (weight without legs, head, intestine, skin and feathers), deboned breast meat, abdominal fat, and thighs.

2.2.2.2. Viscosity measurement

Stored samples were thawed overnight, centrifuged using a Thermo Scientific autoclavable 138 °C Heraeus Megafuge 16r centrifuge at 10,000× g RPM speed and 25 °C adjusted temperature for 10 min (Thermo Fisher Scientific, Waltham, MA, USA). Viscosity analysis was conducted with a programable digital Wells-Brookfield LVDV-II+Pro Cone/Plate Viscometer (Brookfield Engineering, Waltham, MA, USA) with LCD display output to connect a PC.

2.2.2.3. Determination of total N, NH4-N, and uric acid-N contents of the excreta samples

From the excreta samples the dry matter (DM), total N, NH_4-N, and uric acid-N contents were also determined. Total N was analysed using Kjeldahl method using Foss-Kjeltec 8400 analyser unit (Nils Foss Alle 1, Hilleroed, Denmark). NH_4-N and uric acid-N were measured according to Peters et al. (2003)and Marquardt et al. (1983), respectively. The urinary N content was calculated as the sum of the NH_4-N + uric acid N. The faecal N content was calculated as the difference between total N and urinary N.

declared at $p < 0.05$.

2.2.3. Statistical analysis

All statistical analyses were carried out by the software package SPSS 29.0 for Windows, (IBM Corp. in Armonk, NY. USA), using a completely randomized design. The pens were the experimental units for the production traits. During the

digestibility trial birds were moved from the pens to individual balanced cages. In this case the experimental units were cages containing two birds during the grower phase and one bird per cage during the finisher phases. The averages of tested parameters were analysed by one-way analysis of variance (ANOVA) and a general linear model for univariates for parameter with interaction effects such as viscosity, and AA digestibility. Normality of was assessed using Shapiro–Wilk test and Q-Q plots, and homogeneity of variance using Levene test. Significant differences between groups were tested by Tukey HSD post hoc test and if the distribution of data was not homogenous, Games-Howel and Welch tests were applied. Statistical significance has been declared at $p < 0.05$.

2.3. Materials and methods of the second experiment

Though several studies have examined the beneficial effects of high doses of phytase enzyme on production parameters, but there is little research on sunflower meal. Therefore, the aim of the second experiment is to examine whether extra phytase enzyme supplementation to the soybean-corn-wheat based diet can show further improvements in the production traits, nutrient digestibility, nitrogen retention, metabolizable energy content, length of the small intestine, relative organ weights, gut content pH, jejunal histomorphology and gut microbiota.

2.3.1. Experimental diets

Four iso-nitrogenous and iso-caloric diets were formulated according to breeder's recommendations (Aviagen, 2018). The dietary treatments consisted of a soybean-wheat-corn based control diet (C) containing NSP degrading enzyme (Natugrain® TS, 0.1g/kg) and phytase (500 FTU), the C diet supplemented with an extra phytase (CP) (1500 FTU), sunflower meal-based diet (SM), and the SM-based-diet supplemented with extra phytase (SMP) (1500 FTU). The sunflower meal used in this experiment contained, 35.5% CP, and 19% CF.

During the starter phase (days 0 to 11), all birds were fed common starter diet without SM. In the grower (day 11 to 24) and finisher (day 25 to 42) diets, SM was incorporated into the diets at 20% and 30%, respectively. Sunflower meal mainly replaced soybean meal and corn. To compensate the low energy content of SM, sunflower oil was added to the diets. The measured nutrient contents were close to the predicted values (Table 4).

Table 3. Composition of the experimental diets (g/kg)

Ingredients	Starter	Grower				Finisher			
		C	CP	SM	SMP	C	CP	SM	SMP
Corn	392.0	472.0	472.0	363.0	363.0	533.0	533.0	370.0	370.0
Wheat	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0
Extracted soybean meal	400.0	324.0	324.0	193.0	193.0	267.0	267.0	71.0	71.0
Extracted sunflower meal	0.0	0.0	0.0	**200.0**	**200.0**	0.0	0.0	**300.0**	**300.0**
Sunflower oil	55.0	56.0	56.0	94.0	94.0	54.0	54.0	111.0	111.0
MCP [1]	16.0	15.0	15.0	15.0	15.0	13.0	13.0	14.0	14.0
Limestone	18.0	15.0	15.0	14.0	14.0	13.0	13.0	11.0	11.0
Premix[2]	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0
Salt	3.0	3.0	3.0	3.0	3.0	3.0	3.0	3.0	3.0
Sodium bicarbonate	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
Lysine	4.0	4.0	4.0	6.0	6.0	5.0	5.0	8.0	8.0
Methionine	4.0	3.0	3.0	3.0	3.0	3.0	3.0	3.0	3.0
Threonine	1.0	1.0	1.0	2.0	2.0	2.0	2.0	2.0	2.0
Valine	1.0	0.5	0.5	0.5	0.5	1.0	1.0	1.0	1.0
Natugrain® TS[3]	0.0	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1
Phytase (Quantum Blue)[4]	0.0	0.1	**0.3**	0.1	**0.3**	0.1	**0.3**	0.1	**0.3**
Coccidiostat (600g/t)	0.0	0.6	0.6	0.6	0.6	0.0	0.0	0.0	0.0
Total (g)	1000	1000	1000	1000	1000	1000	1000	1000	1000

C- control, CP—C + phytase, SM – Sunflower meal, SMP – SM + phytase, [1]Monocalcium phosphate; [2]Premix was supplied by Agrofeed Ltd. (Győr, Hungary). The active ingredients contained in the premix were as follows (NE per kg of diet): vitamin A—2,000,000 NE, vitamin D3—600,000 NE, vitamin E—6,000mg, menadione—400mg, thiamine—436mg, riboflavin—1,200mg, pyridoxin HCl—600mg, cyanocobalamin—4mg, niacin—6,254mg, pantothenic acid—1825mg, folic acid—300mg, biotin—30mg, betaine—30,000mg, BHT—79.5mg, BHA— 79.5mg, citric acid—71.5mg, Zn (as ZnO)—8,000mg, Zn (as 3b607)—8,000mg, Cu (as 3b413)—2,000mg, Fe (as FeSO4H2O)—10,000mg, Mn (as MnO)—10,000mg, Mn (as 3b506)—10,000mg, I [as Ca(IO3)2]—300mg, Se (as C5H11NO2Se)—40mg, endo-1.4-beta-xylanase—244,000U, Endo-1.3(4)-beta-glucanase—30,400U, 6-phytase—100,000 FTU. [3]Natugrain® TS (100g/1000kg, 0,1g/kg) contains BASF's highly purified NSP-degrading enzymes endo-1, 4-β-xylanase and endo-1,4-β-glucanase. [4]Phytase (Quantum® Blue: 500FTU=100g/1000hg; 1500 FTU= 300g/1000kg).

Table 4. Measured nutrient content of the experimental diets (g/kg)

Nutrients	Starter	Grower				Finisher			
		C	CP	SM	SMP	C	CP	SM	SMP
Dry matter	898.1	895.1	899.3	906.8	904.8	893.0	887.1	898.8	897.1
Crude protein	244.7	199.1	200.6	207.0	206.2	187.8	182.0	187.1	188.6
Crude fat	73.1	72.8	73.0	102.5	107.5	75.2	74.1	120.0	124.4
Crude fiber	41.7	44.0	39.8	92.2	93.0	47.5	46.3	97.8	98.6
Ash	72.7	59.9	59.1	64.3	64.2	64.3	57.9	63.9	67.1
Calcium	10.4	9.7	09.9	10.1	9.8	9.1	8.8	8.9	9.2
Phosphorus	7.7	7.0	07.2	7.4	7.4	6.4	6.4	6.5	6.6
Starch	327.7	401.7	403.2	327.2	324.2	416.4	421.4	318.4	307.3
GE[1] (KJ/g)	175.2	176.3	178.6	185.2	186.1	179.2	180.3	187.4	188.4
NDF[2]	137.8	139.3	138.5	157.2	157.9	140.2	142.9	167.2	166.1
IDF[3]	131.7	125.9	123.7	152.5	154.1	114.7	115.2	166.7	163.2
SDFP[4]	36.9	32.1	34.4	36.5	35.3	29.3	29.0	40.7	38.9
ARG	16.0	12.9	13.0	14.1	14.4	11.7	11.5	13.6	13.4
ILE	10.1	8.3	8.2	09.1	8.4	7.5	7.3	7.1	7.1
LYS	15.5	12.5	12.4	12.7	12.7	11.8	11.7	11.8	11.7
MET	7.1	5.5	5.6	05.8	5.8	5.2	5.2	5.8	5.8
THR	9.9	8.0	8.5	09.3	9.6	8.6	8.3	8.8	8.7
VAL	12.2	9.6	9.4	09.8	10.1	9.4	9.1	9.6	9.7

C—control, CP—C + Phytase, SM—Sunflower meal, SMP—SM + Phytase, [1]Gross energy, [2]Nuetral detergent fibre, [3]Insoluble dietary fibre, [4]Soluble dietary fibre precipitable, ARG—arginine, HIS—histidine, ILE—isoleucine, LEU—leucine, LYS—lysine, MET—methionine, PHE—Phenylalanine, THR—threonine, VAL—valine, CYS—cystine, TYR—tyrosine.

2.3.2. Samplings and measurements

2.3.2.1. Production traits and digestibility trial

Body weight (BW) and feed intake (FI) were measured at the end of the grower (day 24) and finisher (day 36) phases. Body weight gain (BWG) was calculated by deducting the average initial body weight from the average final body weight. Feed conversion ratio (FCR) was calculated based on grams of feed consumed to produce a gram of weight gain.

On day 36, a total of 32 birds, 8 from each treatment, were moved and randomly placed into 32 individual balanced cages. Automatic drinkers and feeders were fixed to each cage, and the chickens were fed their finisher diets containing titanium dioxide (TiO2) as an indigestible marker at a rate of 5 g/kg. On day 41 and 42 representative excreta samples were collected. On the second day, the daily samples were thoroughly homogenised, frozen and stored until analysis. On day 42, all experimental animals were euthanised and slaughtered according to national animal welfare legislation (Hungarian Government Decree 40/2013). The abdominal cavity of the animals was opened immediately, and the left caecal sack content and 2/3 of the distal ileum digesta were collected. The gut contents

were gently squeezed and poured into Eppendorf tubes (0.5 mL) and stored in a refrigerator at −20 °C before AA, and SCFA analyses.

Empty organ weight measurements of the gizzard, ceca, liver, heart, spleen, and bursa fabricus were also performed and expressed as its percentage of the body weight. The length of the duodenum, jejunum, and ileum as wellas whole small intestine (as a sum of the three parts) was also measured and expressed relative to body weight (cm/kg BW).

The pH of the duodenum, jejunum and ileum was determined from the fresh digesta contents of each segment. The fresh samples were homogenized, diluted with distilled water (1: 5), and shaken by hand for one minute. The pH was measured using a SNEX electrode (pH200A), a portable pH meter equipped with a CS1068 pH sensor (CLEAN Instruments, Shanghai, China).

2.3.2.2. Histomorphology measurement

About 2 cm long gut sample was taken from the middle of jejunum and stored in formalin until analysis. After processing, which consisted of serial dehydration, clearing, paraffin tissue stabilization, wax impregnation, and formation of wax blocks, tissue sections were cut at 5 µm thickness from each of the six replicates. The sections were prepared using a Microm HM 360 rotary microtome (Microm International GmbH, Robert-Bosch Str. 49, Walldorf, Germany) and mounted on labelled slides. A routine staining procedure was then performed by immersing the tissue slides in solutions containing nuclear stains and graded ethanol concentrations, followed by haematoxylin and eosin staining.

The prepared slides were examined under an Olympus BX43 microscope fitted with an Olympus DP26 digital video camera (Olympus Corporation, Tokyo, Japan) at 20× magnification (40 × 0.5). The images were analysed using ImageJ software (version 1.47) developed by the National Institute of Health (Bethesda, MD, USA). Well-oriented villus-crypt units were selected from each intestinal-cross section then Villus height (VH), crypt depth (CD), Villus width (VW), and Muscle layer thickness (MLT) were measured. For each section, the villus height to crypt ratio (VH/CD) was calculated. Villus surface area (VSA) was also determined as follows: $\mathrm{VSA} = 2 \times 3.14\ (\mathrm{VW}/2) \times \mathrm{VH}$

2.3.2.4. DNA extraction, PCR Amplification of the 16S rRNA genes, Illumina MiSeq sequencing, bioinformatic analyses

The method of DNA extraction, 16S rRNA gene amplification and Illumina MiSeq Sequencing were implemented based on the description of Such et al. (2023). The microbiome analysis was performed using the Quantitative Insights Into Microbial Ecology 2 (QIIME2—version 2020.2) software (Estaki et al., 2020). The raw sequence data were demultiplexed and filtered using the q2—

demux plugin, followed by denoising with Deblur (Amir et al., 2017). Sequences were filtered based on the QIIME2 default setting. The sequences were clustered into operational taxonomic units (OTUs) using the VSEARCH centroid-based algorithm. The SILVA database release (ver. 132) was used as a reference for taxonomic assignment at a similarity level of 97% (Quast et al., 2012). Alpha and beta diversity were estimated using the QIIME2 diversity plugin and MicrobiomeAnalyst (https://www.microbiomeanalyst.ca/, accessed on 1 August 2024) online software after the data were rarefied to 10,000 sequences/sample (Chong et al., 2020). Raw sequence data of 16S rRNA metagenomics analysis were deposited in the National Center for Biotechnology Information (NCBI) Sequence Read Archive under the BioProject identifier PRJNA1168431.

2.3.3. Statistical Analysis

All statistical analyses were carried out by the software package SPSS 29.0 for Windows, (IBM Corp. in Armonk, NY. USA), using a completely randomized design. The pens were the experimental units for the production traits. During the digestibility trial birds were moved from the pens to individual balanced cages. In this case the experimental units were cages containing two birds during the grower phase and one bird per cage during the finisher phases. The averages of the tested parameters were analysed using two-way ANOVA (2 x 2 factorial, full factorial model) via a general linear model for univariate analysis, using the dietary and enzyme treatments as main factors, and their interaction. Normality of model residuals was assessed using Shapiro–Wilk test and Q-Q plots, and homogeneity of variance using Levene test. However, in the case of experiment two no post hoc test was applied as each factor consisted only two levels. Statistical significance has been declared at $p < 0.05$.

The microbiota analysis was performed with MicrobiomeAnalyst web-based tools filtered for low abundance sequences (<0) based on the mean abundance of OTUs, and for low variability (<10%) using interquartile range assessment. After being filtered, OTU abundances were not rarefied and transformed. To examine within-sample diversity, the α-diversity indices, including Chao1, Shannon, and Simpson indices were calculated. To investigate differences in the structure of microbial communities the principal coordinate analysis (PCoA) was performed, based on the β-diversity index using PERMANOVA (permutational analysis of variance) based on the Bray–Curtis dissimilarity matrix and Unweighted Unifrac Distance. To verify the significance of the bacterial community one-way analysis of variance (ANOVA) was used with a Benjamini-Hochberg False discovery rate-adjusted p-value less than 0.05 were considered as statistically significant. Abundances of microbial taxa were expressed as percentages of total 16S rRNA gene sequences.

3. RESULTS AND DISCUSSION

3.1. First experiment

3.1.1. Production traits

No significant difference was observed in FI and BWG during the grower phase among all treatment groups, but both HFSM and LFSM resulted in significant higher FCR compared with the C-diet. Unexpectedly, significantly higher BWG was recorded in the finisher phase at the HFSM30 treatment. Feeding the LFSM decreased BWG at 30% significantly, compared with the control. The same tendency was found in the finisher phase, but in this case only the LFSM20 treatment caused significantly higher FCR.

Table 5. The effect of dietary treatments on the production traits (mean ± SEM)

Parameters	Treatment	Grower Phase (Days 11–24)	Finisher Phase (Days 25–38)	Overall Mean (Days 0–38)
FI (g/bird)	C	1084.33 ± 32.08	2100.91 ± 138.46	3185.24 ± 158.23
	HFSM20	1137.93 ± 31.41	2205.36 ± 59.25	3343.28 ± 89.40
	HFSM30	1105.41 ± 20.12	2270.53 ± 22.55	3375.93 ± 36.17
	LFSM20	1152.94 ± 18.45	2162.95 ± 64.83	3315.89 ± 81.92
	LFSM30	1119.34 ± 21.63	2086.25 ± 73.94	3205.59 ± 90.93
	p-value	0.375	0.495	0.581
BWG (g)	C	858.14 ± 32.65	1547.10 ± 63.96 ^{b}	2405.24 ± 90.46
	HFSM20	835.74 ± 24.26	1553.57 ± 29.96 ^{b}	2389.31 ± 52.82
	HFSM30	815.14 ± 11.87	1603.41 ± 32.22 ^{a}	2418.55 ± 38.29
	LFSM20	848.20 ± 22.81	1430.40 ± 36.39 ^{bc}	2278.60 ± 58.42
	LFSM30	824.81 ± 23.34	1389.32 ± 42.80 ^{c}	2214.13 ± 62.30
	p-value	0.719	0.023	0.125
FCR (g/g)	C	1.27 ± 0.01 ^{b}	1.36 ± 0.07 ^{b}	1.32 ± 0.04 ^{b}
	HFSM20	1.36 ± 0.02 ^{a}	1.42 ± 0.02 ^{ab}	1.40 ± 0.02 ^{ab}
	HFSM30	1.36 ± 0.01 ^{a}	1.42 ± 0.02 ^{ab}	1.40 ± 0.01 ^{ab}
	LFSM20	1.36 ± 0.02 ^{a}	1.51 ± 0.03 ^{a}	1.46 ± 0.02 ^{a}
	LFSM30	1.36 ± 0.02 ^{a}	1.50 ± 0.02 ^{ab}	1.45 ± 0.01 ^{a}
	p-value	0.001	0.036	0.008

C—control diet, HFSM—high-fibre sunflower meal, LFSM—low-fibre sunflower meal, HFSM20—diet that contain 20% HFSM, HFSM30—diet that contain 30% HFSM, LFSM20—diet that contain 20% low-fibre sunflower meal, LFSM30—diet that contain 30% LFSM, FI—Feed intake, BWG —Body weight gain, FCR—Feed conversion ratio, ^{a,b,c} means with different superscripts in the same column are significantly different ($p < 0.05$).

The responses depend on the fibre content of SM and the parameters investigated. HFSM increased FI and, surprisingly, also increased BWG at 30% in the finisher phase, possibly due to the more developed digestive system of older broilers,

which can better tolerate high fibre levels. Since both FI and BWG increased, HFSM did not modify the FCR compared with the C treatment.

3.1.2. Effects of dietary treatment on carcass composition

Sunflower meal treatments did not affect the relative carcass, breast meat, and thigh weights ($p>0.05$). The abdominal fat percentage, however, increased when the LFSM-containing diets were fed. The difference between the LFSM20 and HFSM30 groups was significant ($p=0.03$). The abdominal fat percentage of birds fed LFSM20 was 54.24% higher than the birds fed HFSM30. The reason could be, that the LFSM diets contained more starch (LFSM20 37.61%; HFSM30 30.98%), but less fat (LFSM20 9.83%; HFSM30 13.10%). The differences in digestion dynamics and the absorption of fatty acids and glucose could be the potential reason for the changes of abdominal fat.

Based on the current study results, it can be concluded that SM has only limited effect on carcass traits and abdominal fat percentage, if the diets are isocaloric and balanced in essential amino acids.

3.1.3. Dietary treatment effects on nutrient digestibility, dietary metabolizable energy content and nitrogen retention

The treatments resulted in nutrient dependent changes in the digestibility. All SM-containing diets decreased the digestibility of starch in comparison with the control significantly, except for LFSM20 on day 41. The lowest starch digestion belonged to the group. The traditional HFSM failed to modify fat digestibility, AMEn and N retention at day 27 and day 41 in comparison with the control treatment. Compared to the control, only LFSM caused significant difference in this trial. The low-fibre SM increased significantly the faecal digestibility of fats and AMEn at both age categories. The treatments affected AME in line with the digestibility of fats. The N retention of birds increased in the LFSM group only during the grower phase.

The results suggest a negative correlation between the fibre intake and starch digestibility, which could be the results of the well-known diluting effects of fibre, which hinders the efficiency of α-amylase. The reason for the opposite trend in fat digestion could be that fibre does not significantly disturb micelle formation in the small intestine (Vivares et al., 2025).

Table 6. Faecal nutrient digestibility, metabolizable energy and nitrogen retention (mean ± SEM)

Treatments	Fat (%)	Starch (%)	AME (kJ/g)	AMEn (kJ/g)	N Retention (%)
			Day 27		
C	91.3 ± 0.24 ^{b}	85.2 ± 0.26 ^{a}	13.9 ± 0.12 ^{c}	13.7 ± 0.12 ^{c}	63.1 ± 1.16 ^{b}
HFSM20	92.0 ± 0.50 ^{abc}	82.1 ± 0.36 ^{bc}	13.7 ± 0.04 ^{c}	13.5 ± 0.04 ^{c}	64.7 ± 0.81 ^{b}
HFSM30	90.5 ± 0.50 ^{bc}	79.0 ± 0.27 ^{d}	13.9 ± 0.06 ^{c}	13.7 ± 0.06 ^{c}	63.6 ± 1.02 ^{b}
LFSM20	93.5 ± 0.16 ^{a}	83.0 ± 0.17 ^{b}	14.5 ± 0.08 ^{b}	14.3 ± 0.08 ^{b}	66.1 ± 0.66 ^{ab}
LFSM30	93.7 ± 0.34 ^{a}	81.4 ± 0.32 ^{c}	15.2 ± 0.12 ^{a}	15.0 ± 0.12 ^{a}	69.4 ± 0.96 ^{a}
p-value	<0.001	<0.001	<0.001	<0.001	<0.001
			Day 41		
C	88.1 ± 0.53 ^{b}	82.9 ± 0.62 ^{a}	13.4 ± 0.30 ^{bc}	13.2 ± 0.30 ^{bc}	64.0 ± 1.52
HFSM20	84.4 ± 0.42 ^{c}	79.9 ± 0.74 ^{bc}	13.5 ± 0.32 ^{bc}	13.3± 0.32 ^{bc}	63.6 ± 1.24
HFSM30	87.5 ± 0.51 ^{b}	76.3 ± 0.43 ^{d}	13.4 ± 0.55 ^{c}	13.1 ± 0.55 ^{c}	62.3 ± 1.50
LFSM20	88.5 ± 0.64 ^{b}	81.3 ± 0.61 ^{ab}	14.0 ± 0.47 ^{ab}	13.8 ± 0.46 ^{ab}	66.9 ± 1.61
LFSM30	92.2 ± 0.42 ^{a}	78.3 ± 0.60 ^{cd}	14.2 ± 0.44 ^{a}	13.9 ± 0.42 ^{a}	65.7 ± 2.83
p-value	<0.001	<0.001	<0.001	<0.001	0.424

C—control diet, HFSM—high-fibre sunflower meal, LFSM—low-fibre sunflower meal, HFSM20—diet that contain 20% HFSM, HFSM30—diet that contain 30% HFSM, LFSM20—diet that contain 20% low-fibre sunflower meal, LFSM30—diet that contain 30% LFSM, ^{a,b,c,d} means with different superscripts in the same column are significantly different ($p < 0.05$). AME—apparent metabolisable energy, AMEn—apparent metabolisable energy nitrogen corrected.

3.1.4. Ileal amino acid digestibility on day 27

The apparent ileal AA digestibility values at day 27 are shown in Table 7. The inclusion of HFSM and LFSM increased the digestibility of most of the AAs. Compared with the control treatment both HFSM inclusion rates increased the digestibility of ARG and THR. Feeding HFSM at 30% did not result in depression of the AA absorption, or in the case of ILE and CYS, even resulted in further increase compared with the HFSM20 treatment. The difference between the HFSM and control group was significant for ARG, ILE, THR, TYR and CYS. LEU was the only amino acid of which digestibility decreased if HFSM was fed. The LFSM treatments had also a positive impact on AA digestion, LFSM30 in particular improved digestibility of most of the amino acids compared with the control (ARG, HIS, ILE, LYS, MET, THR, VAL, TYR). The absorption of LEU was unaffected by LFSM.

Table 7: Apparent ileal amino acid digestibility on day 27

Amino acids	C	HFSM20	HFSM30	LFSM20	LFSM30	P-value
Arginine	80.0 ^{c}	84.6 ^{ab}	84.5 ^{b}	87.6 ^{ab}	88.0 ^{a}	<0.001
Histidine	77.8 ^{b}	78.6 ^{b}	77.2 ^{b}	81.5 ^{ab}	81.3 ^{a}	0.003
Isoleucine	76.8 ^{b}	77.7 ^{b}	82.5 ^{a}	83.5 ^{a}	83.1 ^{a}	<0.001
Leucine	86.2 ^{a}	83.8 ^{b}	80.6 ^{c}	84.3 ^{abc}	85.1 ^{ab}	<0.001
Lysine	72.3 ^{b}	74.9 ^{ab}	73.9 ^{ab}	77.3 ^{a}	77.4 2^{a}	0.044
Methionine	84.8 ^{bc}	83.5 ^{c}	84.3 ^{c}	87.3 ^{ab}	88.4 ^{a}	<0.001
Phenylalanine	83.9 ^{ab}	82.9 ^{ab}	82.4 ^{b}	85.0 ^{ab}	85.45 ^{a}	0.022
Threonine	58.3 ^{b}	67.0 ^{a}	69.9 ^{a}	70.3 ^{a}	69.6 ^{a}	<0.001
Valine	74.6 ^{b}	79.2 ^{a}	78.1^{ab}	82.1 ^{a}	81.9 ^{a}	<0.001
Tyrosine	71.8^{c}	75.4 ^{bc}	77.3 ^{b}	83.2 ^{a}	82.9 ^{a}	<0.001
Cystine	74.7 ^{bc}	74.4 ^{c}	78.6 ^{a}	78.1 ^{ab}	77.7 ^{abc}	0.003

HFSM—High-fibre sunflower meal, LFSM—Low-fibre sunflower meal, HFSM20—diet that contains 20% HFSM, HFSM30—diet that contains 30% HFSM, LFSM20—diet that contains 20% LFSM, LFSM30—diet that contains 30% LFSM, ^{a,b,c} means with different superscripts in the same column are significantly different ($p < 0.05$).

3.1.5. Amino acid digestibility on day 41

3.1.5.1. Amino acid digestibility in the distal jejunum

The apparent amino acids digestibility values in the distal jejunum at day 41 are presented in Table 8. Compared with the control diet, feeding HFSM at both inclusion rates improved the digestibility of THR and TYR digestibility. HFSM20 resulted also in a significant increase in the case of ILE, while HFSM30 in the case of ARG and MET. The effect of LFSM in this gut section was low; the differences were significant only for MET (LFSM20, LFSM30), THR (LFSM20) and TYR (LFSM30).

Table 8: Apparent amino acids digestibility in the distal jejunum on day 41

Amino acids	C	HFSM20	HFSM30	LFSM20	LFSM30	P-value
Arginine	70.0 ^{b}	72.3 ^{ab}	75.6 ^{a}	74.9 ^{ab}	73.6 ^{ab}	0.019
Histidine	63.6	68.7	67.0	65.5	67.5	0.226
Isoleucine	64.3^{b}	71.3 ^{a}	69.5 ^{ab}	64.2 ^{b}	65.2 ^{b}	<0.001
Leucine	73.6	72.9	71.9	71.6	69.6	0.237
Lysine	56.7	61.2	61.0	62.8	63.5	0.085
Methionine	67.1 ^{b}	73.6 ^{ab}	74.7 ^{a}	74.5 ^{a}	74.6^{a}	0.029
Phenylalanine	72.8	73.5	72.3	69.3	72.4	0.227
Threonine	44.8 ^{c}	58.6 ^{a}	54.5 ^{ab}	55.9 ^{ab}	50.3^{bc}	<0.001
Valine	63.6	68.2	64.8	66.2	65.8	0.366
Tyrosine	56.7	62.5	62.1	60.6	62.8	0.161
Cystine	58.7 ^{b}	69.6 ^{a}	65.4 ^{a}	63.6 ^{ab}	68.5^{a}	<0.001

HFSM—High-fibre sunflower meal, LFSM—Low-fibre sunflower meal, HFSM20—diet that contains 20% HFSM, HFSM30—diet that contains 30% HFSM, LFSM20—diet that contains 20% LFSM, LFSM30—diet that contains 30% LFSM, ^{a,b,c} means with different superscripts in the same column are significantly different ($p < 0.05$).

3.1.5.2 Amino acid digestibility in the proximal ileum

The effects of feeding SM on the apparent AA digestibility in the proximal ileum are shown in Table 9. Both HFSM treatments increased the digestibility of LYS, THR, and TYR, while decreased that of LEU. High fibre SM at 30% resulted further improvement in the case of ARG and ILE. Interestingly, the inclusion of LFSM at 20% and 30% significantly enhanced the digestibility of all amino acids except that of LEU.

Table 9: Apparent amino acid digestibility in the proximal ileum on day 41

Amino acids	C	HFSM20	HFSM30	LFSM20	LFSM30	P-value
Arginine	80.4 ^{c}	82.8 ^{bc}	83.7 ^{b}	88.8 ^{a}	87.9^{a}	<0.001
Histidine	75.2 ^{b}	76.8 ^{b}	77.4 ^{b}	82.4 ^{a}	82.5 ^{a}	<0.001
Isoleucine	77.3 ^{d}	79.3 ^{cd}	80.6 ^{bc}	82.8 ^{ab}	83.6 ^{a}	<0.001
Leucine	85.2 ^{a}	82.1^{b}	81.6 ^{b}	84.9 ^{a}	85.4 ^{a}	<0.001
Lysine	68.5 ^{c}	72.7 ^{b}	74.2 ^{ab}	77.3 ^{a}	77.4 ^{a}	<0.001
Methionine	84.2 ^{b}	84.0 ^{b}	85.1 ^{b}	88.4 ^{a}	88.5^{a}	<0.001
Phenylalanine	82.5 ^{b}	82.6 ^{b}	82.6 ^{b}	85.2 ^{a}	85.4 ^{a}	<0.001
Threonine	58.0 ^{b}	67.9 ^{a}	65.4 ^{a}	70.4 ^{a}	69.2 ^{a}	<0.001
Valine	75.2 ^{b}	77.7 ^{b}	78.4 ^{ab}	81.9 ^{a}	81.6 ^{a}	<0.001
Tyrosine	69.6 ^{c}	77.2 ^{b}	78.7 ^{b}	82.7 ^{a}	82.4 ^{a}	<0.001
Cystine	72.7 ^{b}	73.7 ^{b}	74.7 ^{b}	79.3^{a}	77.9 ^{a}	<0.001

HFSM—High-fibre sunflower meal, LFSM—Low-fibre sunflower meal, HFSM20—diet that contains 20% HFSM, HFSM30—diet that contains 30% HFSM, LFSM20—diet that contains 20% LFSM, LFSM30—diet that contains 30% LFSM, ^{a,b,c,d} means with different superscripts in the same column are significantly different ($p < 0.05$).

3.1.5.3 Amino acid digestibility in the distal ileum

The results are shown in Table 10. Mainly the same amino acids were affected as in the previous ileal part. High fibre SM increased ARG, THR, VAL, and TYR. In addition, HFSM20 increased LYS digestibility. Low fibre SM increased digestibility of all amino acids except LEU. Again, the effect of HFSM on leucine digestibility was negative while unaffected by LFSM.

Feeding SM based diets at 10%, 20% and 30% increased the ileal AA digestibility of THR, VAL, LYS, ARG, and TYR but decreased the digestibility of LEU (Such et al., 2024). Similarly, supplementation of 10-20-30% SM in pullet diets increased the absorption of THR, VAL, LYS, and ARG but impaired the absorption of LEU (Mezőlaki, 2024). Though the experimental animals were layer pullets, their results support the current findings.

On day 41, in the jejunal part there was an increase in TRH and TYR (HFSM), ILE (HFSM20), and ARG and MET (HFSM30). However, the effect of LFSM was low; it increased only the digestibility of MET, THR and TYR (Table 8), and the distal jejunal AA digestibility relative to distal ileal AA digestibility was also higher when HFSM was fed (Table 13).

Table 10: Apparent amino acids digestibility in the distal ileum on day 41

Amino acids	C	HFSM20	HFSM30	LFSM20	LFSM30	P-value
Arginine	81.3 ^{c}	84.1 ^{b}	86.1 ^{ab}	87.7 ^{a}	87.3 ^{a}	<0.001
Histidine	77.9 ^{b}	79.2 ^{ab}	77.9 ^{b}	81.5 ^{a}	81.6 ^{a}	<0.001
Isoleucine	80.9 ^{b}	79.7 ^{b}	79.5 ^{b}	83.8 ^{a}	84.0 ^{a}	<0.001
Leucine	87.1 ^{a}	83.5 ^{bc}	82.4 ^{c}	85.4 ^{a}	85.3 ^{ab}	<0.001
Lysine	71.3 ^{b}	75.9 ^{a}	72.5 ^{b}	76.6 ^{a}	76.3 ^{a}	<0.001
Methionine	85.2 ^{b}	84.7 ^{b}	85.7 ^{b}	89.7 ^{a}	88.2 ^{a}	<0.001
Phenylalanine	83.7 ^{b}	84.1 ^{b}	82.4 ^{b}	86.1 ^{a}	86.4 ^{a}	<0.001
Threonine	58.8 ^{b}	67.9 ^{a}	66.1 ^{a}	71.3 ^{a}	68.3 ^{a}	<0.001
Valine	77.2 ^{d}	80.5 ^{bc}	79.1 ^{c}	83.4 ^{a}	82.3 ^{ab}	<0.001
Tyrosine	71.9 ^{c}	81.3 ^{ab}	79.7 ^{b}	83.2 ^{a}	81.3 ^{ab}	<0.001
Cystine	73.3 ^{c}	74.2 ^{c}	75.2 ^{bc}	81.0 ^{a}	77.7 ^{ab}	<0.001

HFSM—High-fibre sunflower meal, LFSM—Low-fibre sunflower meal, HFSM20—diet that contains 20% HFSM, HFSM30—diet that contains 30% HFSM, LFSM20—diet that contains 20% LFSM, LFSM30—diet that contains 30% LFSM, ^{a,b,c} means with different superscripts in the same column are significantly different ($p < 0.05$).

This indicates that feed containing more fiber was better digested compared with feed containing less fibre in the jejunal part of the small intestine. There is no research directly compared the effects of LFSM in broiler chickens, and the

effects of both HFSM and LFSM on the dynamics of AA digestibility. However, our results revealed that feeding LFSM increased the digestibility of all amino acids compared with the C diet while LEU was unaffected.

Though HFSM diets improved the digestibility of some of the AAs in comparison with the C diet, most importantly, LFSM improved the digestibility of all AAs. Even the negatively affected LEU digestibility when animals were fed with HFSM, remain unaffected after the animals were fed with LFSM. Sunflower meal was incorporated in the isocaloric and isonitrogenous diet, as a result, it is clear that the improvement in digestibility was the effect of fiber. Slightly reducing the fiber content of SM further improved the digestibility of the amino acids. As a result, LFSM should be used for improved AA digestibility of the broiler diet.

The inclusion of IDF up to 50 g/kg DM in a standard cereal-soybean based diet stimulates the development and function of digestive organs such as gizzard and thus improves the digestibility of nutrients to levels up to 10% to 12% (Vivares et al., 2025). Therefore, the IDF content of HFSM (34%) and LFSM (24.9%) in the current study could be the reason for the better AA digestibility of the broilers, though SM was included up to 30% of the broiler diet.

In this experiment HFSM decreased the digestibility of leucine consequently. It was the only amino acid of which digestibility impaired. The reason for this could be that HFSM increased the endogenous protein losses (mucins, enzymes, sloughed epithelial cells) in the small intestine. The endogenous secretions are rich in branched chain amino acids, that decrease the apparent digestibility of leucine, even when the true digestibility is unchanged. This fact was supported by Cerrate et al. (2018), they investigated the effects of dietary nutrients on the ileal endogenous losses of threonine, cystine, methionine, lysine, and leucine. They found that increasing dietary NDF from 11.5% to 18% significantly increased the endogenous losses of leucine only, after feeding to male broilers at 21 days of age. Ravindran (2021) also reported a similar information.

In conclusion, based on the ileal and jejunal digestibility of AAs it is clear that both HFSM and LFSM up to 30% inclusion level increased the digestibility of most of the AAs, except LEU.

3.1.6. Dietary treatment and intestinal segment effects on the digestibility of amino acids

The two-factorial evaluation results are shown in Table 11. The dietary treatment main effects are in line with the results of the previous tables. Except LEU, SM treatments increased the AA digestibility values in comparison with the control group. The ileal digestibility values were significantly higher than those of the jejunum. Except VAL and TYR no difference was observed between the proximal and distal ileal digestibility, an indication no further digestion of AA happened in the distal ileal part. The feed and intestinal part interaction was significant for most amino acids. The reason for it was that LFSM30 or HFSM30 resulted in lower distal ileal digestibility compared with the proximal ileal coefficients.

Table 11. Dietary treatment and intestinal segment effects on the digestibility of amino acids on day 41 (%)

Feed (F)	IP	ARG	HIS	ILE	LEU	LYS	MET	PHE	THR	VAL	TYR	CYS
C	Distal jejunum	70	63.7	64.4	73.7	56.7	67.2	72.8	44.9	58.8	58.8	56.7
	Proximal ileum	80.4	75.3	77.3	85.1	68.9	84.2	82.5	58	69.6	69.6	72.7
	Distal ileum	81.3	77.9	80.9	87.1	71.3	85.2	83.7	58.8	71.9	71.9	73.3
HFSM-20	Distal jejunum	72.3	68.7	71.4	72.9	61.2	73.6	73.6	58.6	69.7	69.7	62.5
	Proximal ileum	82.8	76.8	78.6	82.1	72.7	84	82.6	68	77.2	77.2	73.8
	Distal ileum	84.1	79.2	79.7	83.5	75.9	84.7	84.1	67.8	81.3	81.3	74.2
HFSM-30	Distal jejunum	75.6	67	69.5	72	61.1	74.7	72.3	54.6	66.0	66	62.1
	Proximal ileum	83.7	77.4	80.6	81.6	74.2	85.1	82.6	65.4	78.7	78.7	74.7
	Distal ileum	86.1	78	79.5	82.4	72.4	85.7	82.4	66.1	79.7	79.7	75.2
LFSM-20	Distal jejunum	74.9	65.5	64.3	71.7	62.8	74.5	69.3	55.9	63.6	63.6	60.6
	Proximal ileum	88	82.4	82.8	84.8	77.3	88.4	85.2	70.4	82.7	82.7	79.3
	Distal ileum	87.7	81.5	83.8	85.4	76.6	89.7	86.1	71.2	83.2	83.2	81
LFSM-30	Distal jejunum	73.6	67.5	65.3	69.7	63.5	74.6	72.5	50.3	68.5	68.5	62.8
	Proximal ileum	87.9	82.5	83.5	85.4	77.4	88.5	85.4	69.2	82.4	82.4	78
	Distal ileum	87.3	81.6	84	85.3	76.3	88.3	86.4	68.2	81.3	81.3	77.7
Feed (F)	C	77.3^{d}	72.3^{c}	74.2^{b}	82.0^{a}	65.6^{d}	78.6^{c}	79.7^{bc}	53.9^{c}	72.0^{c}	66.7^{c}	67.6^{c}
	HFSM-20	79.7^{c}	74.9^{ab}	76.6^{a}	79.5^{bc}	69.9^{bc}	81.0^{b}	80.1^{abc}	64.8^{ab}	75.5^{ab}	76.0^{ab}	70.2^{b}
	HFSM-30	81.8^{bc}	74.1^{bc}	76.5^{a}	78.7^{c}	69.2^{c}	81.8^{b}	79.1^{c}	62.0^{b}	74.1^{bc}	74.8^{b}	70.7^{b}
	LFSM-20	83.6^{a}	76.5^{a}	76.9^{a}	80.6^{ab}	72.2^{a}	84.2^{a}	80.2^{ab}	65.8^{a}	77.2^{a}	76.5^{a}	73.6^{a}
	LFSM-30	82.9^{ab}	77.2^{a}	77.6^{a}	80.1^{abc}	72.4^{ab}	83.8^{a}	81.4^{a}	62.6^{b}	76.5^{ab}	77.4^{a}	72.8^{ab}
Intestinal part (IP)	Distal jejunum	73.3^{b}	66.5^{b}	67.0^{b}	72.0^{b}	61.1^{b}	72.9^{b}	72.1^{b}	52.9^{b}	65.7^{c}	65.3^{c}	61.0^{b}
	Proximal ileum	84.6^{a}	78.9^{a}	80.6^{a}	83.8^{a}	74.1^{a}	86.0^{a}	83.6^{a}	66.2^{a}	78.9^{b}	78.1^{b}	75.7^{a}
	Distal ileum	85.3^{a}	79.6^{a}	81.6^{a}	84.7^{a}	74.5^{a}	86.7^{a}	84.5^{a}	66.4^{a}	80.5^{a}	79.5^{a}	76.3^{a}
	SEM	0.60	0.65	0.69	0.59	0.69	0.65	0.56	0.81	0.69	0.75	0.75
P-Values	Feed	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	0.009	<0.001	<0.001	<0.001	<0.001
	Intestinal part	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
	F x IP	0.051	0.005	<0.001	0.002	0.486	<0.001	<0.001	0.049	0.102	<0.001	0.059

HFSM—High-fibre sunflower meal, LFSM—Low-fibre sunflower meal, HFSM20—diet that contains 20% HFSM, HFSM30—diet that contains 30% HFSM, LFSM20—diet that contains 20% LFSM, LFSM30—diet that contains 30% LFSM, ARG—arginine, HIS—histidine, ILE—isoleucine, LEU—leucine, LYS—lysine, MET—methionine, PHE—phenylalanine, THR—threonine, VAL—valine, TYR—tyrosine, CYS—cystine, ^{a,b,c,d} means with different superscripts in the same column are significantly different ($p < 0.05$).

It was clear in these results, that AA digestibility in the finisher phase was higher due to the fibre contained in SM meal. It was also visible that birds were sensitive to high fibre content in their diet because of their digestive system lack the enzymes to efficiently digest fiber. As a result, feeding LFSM compared with HFSM containing diets showed better AA digestibility.

3.1.7. Dietary treatment and age effects on the ileal AA digestibility

The feed and age interaction effects on the digestibility of amino acids are presented in Table 12. In this case the AA digestibility values were calculated only from the total ileal contents of both age categories. Compared with the control, LFSM enhanced the ileal digestibility of all AAs while HFSM increased ARG, ILE, LYS, THR, VAL and TYR. High fibre SM decreased the absorption rate of LEU.

Birds age has a detrimental effect as the digestive system of the young birds is not well developed to efficiently utilize the fiber rich feed ingredients. The age influence on AA digestibility is feed type and specific AA dependent (Barua et al., 2021). In our experiment, in the case of age effect, only two specific AAs that is MET and TYR digestibility were significantly higher in the 41-day old birds. The feed x age interaction was significant for ILE, CYS and TYR. The interaction was driven by a decrease in digestibility in older chickens when fed HFSM and LFSM diets (ILE in HFSM30 and LFSM20; CYS in C and both HFSMs; TYR in treatment C and LFSM30 diets).

Table 12. Ileal amino acid digestibility of amino acids on day 27 and day 41

Feed	Age	ARG	HIS	ILE	LEU	LYS	MET	PHE	THR	VAL	CYS	TYR
C	Day 27	80.1	77.8	76.8	86.2	72.3	84.8	83.9	58.3	74.6	74.7	71.8
	Day 41	81.0	76.7	79.3	86.1	70.1	84.8	83.1	58.2	76.6	73.0	70.7
LFSM20	Day 27	84.7	78.6	77.8	83.7	74.9	83.5	82.9	67.0	79.2	74.4	75.4
	Day 41	83.4	78.0	79.1	82.8	74.3	84.3	83.3	67.9	79.1	74.0	79.2
HFSM30	Day 27	84.5	77.2	82.5	80.6	74.0	84.3	82.4	69.9	78.1	78.6	77.3
	Day 41	84.9	77.7	80.0	82.0	73.3	85.4	82.5	65.7	78.8	74.9	79.2
LFSM20	Day 27	87.6	81.5	83.5	84.3	77.3	87.3	85.0	70.3	82.1	78.1	83.2
	Day 41	87.9	81.9	83.3	85.1	76.9	89.0	85.6	70.8	82.7	80.2	83.0
LFSM30	Day 27	88.0	81.3	83.0	85.1	77.4	88.4	85.5	69.6	81.9	77.7	82.9
	Day 41	87.6	82.1	83.8	85.3	76.9	88.4	85.9	68.7	81.9	77.8	81.8
Feed	C	80.6^{c}	77.2 ^{b}	78.0^{c}	86.2^{a}	71.2^{c}	84.8^{b}	83.5^{b}	58.2^{b}	75.6 ^{c}	73.9^{c}	71.2^{c}
	HFSM20	84.1^{b}	78.3^{b}	78.5^{c}	83.3^{b}	74.6^{ab}	83.9^{b}	83.1^{b}	67.5^{a}	79.2^{b}	74.2^{c}	77.3^{b}
	HFSM30	84.7^{b}	77.5^{b}	81.3^{b}	81.3^{c}	73.6^{bc}	84.8^{b}	82.5^{b}	67.8^{a}	78.4^{b}	76.8^{b}	78.3^{b}
	LFSM20	87.8^{a}	81.7^{a}	83.4^{a}	84.7^{ab}	77.1^{a}	88.1^{a}	85.3^{a}	70.5^{a}	82.4^{a}	79.1^{a}	83.1^{a}
	LFSM30	87.8^{a}	81.7^{a}	83.4^{a}	85.2^{a}	77.1^{a}	88.4^{a}	85.7^{a}	69.2^{a}	81.9^{a}	77.7^{ab}	82.4^{a}
Age	Day 27	85.0	79.3	80.7	84.0	75.2	85.7^{b}	83.9	67.0	79.2	76.7	78.1^{b}
	Day 41	85.0	79.3	81.1	84.3	74.3	86.4^{a}	84.1	66.3	79.8	76.0	78.8^{a}
SEM	0.37	0.30	0.35	0.25	0.39	0.27	0.22	0.61	0.38	0.36	0.34	SEM
P-Value	Feed	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
	Age	0.969	0.970	0.419	0.400	0.184	0.033	0.688	0.349	0.252	0.158	0.238
	Feed*Age	0.606	0.621	0.011	0.271	0.908	0.365	0.758	0.243	0.766	0.006	0.025

HFSM—High-fibre sunflower meal, LFSM—Low-fibre sunflower meal, HFSM20—diet that contains 20% HFSM, HFSM30—diet that contains 30% HFSM, LFSM20—diet that contains 20% LFSM, LFSM30—diet that contains 30% LFSM, ARG—arginine, HIS—histidine, ILE—isoleucine, LEU—leucine, LYS—lysine, MET—methionine, PHE—phenylalanine, THR—threonine, VAL—valine, TYR—tyrosine, CYS—cystine, ^{a,b,c} means with different superscripts in the same column are significantly different ($p < 0.05$).

3.1.8. Amino acid disappearance rate from the different gut segments

The relative distal jejunum and proximal ileum AA disappearance rates, as the percentage of the distal ileal absorption are shown in Figure 1. It can be seen that the absorption of all amino acids terminated in the proximal ileum, and no further digestion happened in the second part of the ileum. However, the AA absorption dynamics were different. The fastest absorbed AAs in the distal jejunum were ARG, LEU, MET and PHE. The disappearance rates of ILE, LYS, THR, VAL, TYR and CYS were slower.

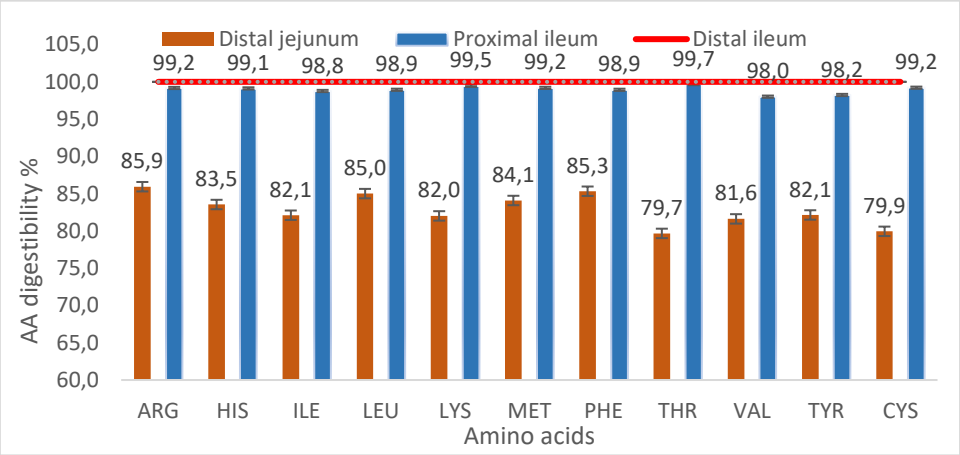

Figure 1. Amino acid disappearance in the distal jejunal and proximal ileal parts relative to the distal ileal AA disappearance

ARG—arginine, HIS—histidine, ILE—isoleucine, LEU—leucine, LYS—lysine, MET—methionine, PHE—phenylalanine, THR—threonine, VAL—valine, TYR—tyrosine, CYS—cystine

3.1.9. Effects of feeding SM on the amino acid absorption dynamics from the distal jejunum

Dietary treatment effects on the distal jejunal relative AA digestibility are presented in Table 13. Feeding HFSM and LFSM increased the speed of MET absorption from the jejunum compared with the control. High fibre SM at both inclusion rates speeded up the jejunal AA digestibility of ILE compared to the C and LFSM diets. The PHE, TYR, and CYS digestibility was also affected by the diets, but the differences in these AAs were significant only between the SM treatments.

Based on the knowledge of the authors, there is no research results about distal jejunum and proximal ileum AA disappearance rates and AA dynamics of SM containing diets. Relative to the distal halves, the effect of fiber on the AA digestibility was evident. The relative AA digestibility of the dietary treatments in the jejunum and proximal ileum was higher for HFSM diets, may be because of higher retention time due to its higher fiber content compare with the C diet and LFSM diets. The digesta passage rate or retention time has a major influence on the digestion and absorption of nutrients. The slower the passage rate, the longer the digesta will be retained in the GIT. This allows more time for contact between digestive enzymes and substrates, as well as between digestion products and the intestinal mucosa (Ravindran and Abdollahi, 2021).

Table 13. Dietary treatment effects on the relative distal jejunal amino acid absorption (%)

Amino acids	Dietary Treatments					
	C	HFSM20	HFSM30	LFSM20	LFSM30	P-value
Arginine	85.1	86.3	87.8	84.7	84.4	0.469
Histidine	81.5	87.2	86.	79.6	82.8	0.137
Isoleucine	79.4 ^{b}	90.3 ^{a}	87.5 ^{a}	76.2 ^{b}	77.7 ^{b}	<0.001
Leucine	84.1	87.6	87.4	83.4	81.7	0.052
Lysine	78.0	81.2	84.4	81.1	83.3	0.484
Methionine	78.1 ^{c}	87.2 ^{a}	87.2 ^{a}	82.7 ^{ab}	84.6 ^{a}	0.001
Phenylalanine	86.3 ^{ab}	87.7 ^{a}	87.8 ^{a}	79.9 ^{b}	83.9 ^{ab}	0.008
Threonine	74.8	84.7	82.7	76.6	73.8	0.063
Valine	81.8	84.9	81.9	78.7	80.1	0.303
Tyrosine	81.4 ^{ab}	85.8 ^{a}	82.8 ^{ab}	76.1^{b}	84.5 ^{a}	0.019
Cystine	75.8 ^{ab}	84.7 ^{a}	82.6 ^{ab}	73.9 ^{b}	80.9 ^{ab}	0.029

HFSM—High-fibre sunflower meal, LFSM—Low-fibre sunflower meal, HFSM20—diet that contains 20% HFSM, HFSM30—diet that contains 30% HFSM, LFSM20—diet that contains 20% LFSM, LFSM30—diet that contains 30% LFSM, ^{a,b,c} means with different superscripts in the same row are significantly different ($p < 0.05$).

3.1.10. Effects of dietary treatment on the dry matter content and nitrogen forms of excreta

According to the findings of the current study, the inclusion of HFSM and LFSM at 30%, reduced the total N excretion of chickens significantly at day 27 (Table 14). Both the NH_4-N and uric acid-N excretion declined in the SM groups, resulting in a significant decrease in the urinary N excretion in the groups of HFSM20, HFSM30, and LFSM20. No significant differences in these parameters were found during the finisher phase, at day 41.

Most of the digestibility trials focus on performance and economic considerations, not mainly focusing on N excretions and ammonia emission reduction (Applegate, 2008). As a result, there are no studies solely on the effects of SM on the N excretion of chickens. Feeding SM-containing diets did not modify the dry matter content of the excreta. According to these results, SM at 20 and 30% does not increase the water content of the excreta. This is positive, since no special exogenous enzymes are available for the degradation of the soluble fibre of SM. At day 41, feeding SM did not affect the N composition of the excreta. On the other hand, the younger chickens excreted less faecal and urinary N, including both NH_4-N and uric acid N.

In birds fed the HFSM30 diet, the uric acid N and the total N excretion was reduced by 18.52% and 15.29%, respectively. It is a positive result from an ammonia emission point of view, since ammonia is formed mainly from the urinary N after bacterial breakdown by urease enzyme (Nahm, 2003; Vilela et al., 2020). The theory behind the decrease is that fibre in the intestine can bind ammonia, reducing the amount that returns back to the liver and, in this way, lowering uric acid synthesis and excretion. In our findings, urinary N was also reduced by 14 to 22% when SM was fed. However, there is no explanation why this mechanism of feeding SM did not work in the older chickens.

Table 14. The dry matter content and the different nitrogen forms of the excreta (% of fresh excreta samples) (mean ± SEM)

Age	Treatment	Faecal DM	NH_4-N	Uric Acid-N	Urinary-N	Faecal-N	Total N
Day 27	C	20.6 ± 0.4	1.0 ± 0.0 ^{a}	2.7 ± 0.1 ^{a}	3.7 ± 0.1 ^{a}	4.8 ± 0.3	8.5 ± 0.4 ^{a}
	HFSM20	21.2 ± 0.6	0.8 ± 0.1 ^{bc}	2.4 ± 0.1 ^{ab}	3.1 ± 0.1 ^{b}	4.2 ± 0.2	7.3 ± 0.3 ^{ab}
	HFSM30	20.7 ± 0.6	0.8 ± 0.0 ^{bc}	2.2 ± 0.1 ^{b}	2.9 ± 0.1 ^{b}	4.3 ± 0.2	7.2 ± 0.2 ^{b}
	LFSM20	20.9 ± 0.3	0.7 ± 0.1 ^{c}	2.4 ± 0.0 ^{ab}	3.1 ± 0.1 ^{b}	4.4± 0.1	7.5 ± 0.2 ^{ab}
	LFSM30	20.8 ± 0.3	0.9 ± 0.1 ^{ab}	2.3 ± 0.2 ^{b}	3.2 ± 0.2 ^{ab}	4.0 ± 0.2	7.2 ± 0.4 ^{b}
	p-value	0.879	0.001	0.004	0.002	0.121	0.028
Day 41	C	18.5 ± 0.5	0.8 ± 0.0	2.1 ± 0.1	3.0 ± 0.1	3.8 ± 0.2	68 ± 0.3
	HFSM20	19.1 ± 0.6	0.8 ± 0.0	1.9 ± 0.1	2.8 ± 0.1	3.2 ± 0.1	60 ± 0.2
	HFSM30	19.5 ± 0.5	0.8 ± 0.0	1.9 ± 0.0	2.6 ± 0.1	3.2 ± 0.1	59 ± 0.2
	LFSM20	18.3 ± 0.6	0.9 ± 0.0	2.0 ± 0.1	2.9 ± 0.1	3.4 ± 0.2	63 ± 0.3
	LFSM30	18.7 ± 0.5	0.8 ± 0.0	2.1 ± 0.1	2.9 ± 0.2	3.3 ± 0.2	62 ± 0.4
	p-value	0.646	0.430	0.222	0.324	0.118	0.156

C—control diet, HFSM—high-fibre sunflower meal, LFSM—low-fibre sunflower meal, HFSM20—diet that contain 20% HFSM, HFSM30—diet that contain 30% HFSM, LFSM20—diet that contain 20% low-fibre sunflower meal, LFSM30—diet that contain 30% LFSM, ^{a,b,c} means with different superscripts in the same column are significantly different ($p < 0.05$)

3.1.11. The jejunal digesta viscosity and caecal SCFA results

Although SM contains soluble fibre fractions, none of the HFSM and LFSM diets increased the viscosity of the jejunal gut contents in comparison with the control diet. Interestingly, feeding HFSM at 30% resulted in the lowest viscosity at both age groups ($p<0.001$). The age and feed interaction effect on digesta viscosity showed that growers had a significantly lower viscosity ($p=0.005$), an indication viscosity increases with age.

An interesting result of this trial was that the HFSM at 30% inclusion level decreased the viscosity in the jejunal content. The reason for the lower viscosity at a higher HFSM level could be due to the higher dietary insoluble fibre content. This may enhance gut motility and reduce digesta viscosity. The same tendency was evidenced at day 41, but in this case the differences between the SM treatments and the control group were not significant. The jejunal content viscosity increased with age. The average viscosity at day 41 was significantly 13.89% higher than that at day 27 ($p<0.001$). The reason for the age-related difference could be that the birds in the finisher phase ate more and, in this way, consumed more soluble dietary fibre, which resulted in increased digesta viscosity in the older chickens. The jejunal viscosity values show negative correlation with the body-weight gain results. The lower viscosity was found at 30% HFSM level, and the highest BWG belonged also to this treatment.

The SCFA content of the caeca was not influenced by the SM treatments. Feeding HFSM or LFSM failed to make a difference in the SCFA content and composition of the caecal content. This means, in the frame of SM digestion, there do not arise such fine and soluble particles that enter the caeca.

3.2. Second experiment

3.2.1. Production Traits

The production performance of the broilers is shown in Table 15. Dietary inclusion of SM and enzyme treatment didn't modify production performance during the grower phase. However, during the finisher phase and the entire experimental period (days 0 to 36), SM at 30% inclusion level increased BWG, and reduced FCR. No effects of using extra phytase was found. Poultry cannot efficiently digest non-starch polysaccharides. To improve nutrient utilization and increase available energy, enzyme supplements are commonly applied.

Table 15. Effects of dietary treatment on the production traits (mean ± SEM)

	Feed (F)	Phytase (P)	FI (g)	BWG (g)	FCR (g/g)
Grower phase (d 11–24)	C	-	1197.8	856.3	1.40
		+	1185.8	853.9	1.38
	SM	-	1188.7	897.6	1.33
		+	1190.0	880.2	1.37
Finisher phase (d 25–36)	C	-	1639.6	974.8	1.67
		+	1743.0	1059.5	1.63
	SM	-	1710.3	1168.6	1.47
		+	1698.9	1148.6	1.50
Overall mean (d 0–36)	C	-	2837.4	1831.1	1.57
		+	2928.8	1913.4	1.53
	SM	-	2901.6	2066.3	1.42
		+	2874.9	2028.8	1.44
Grower phase	Feed	C	1191.8 ± 12.8	855.1 ± 12.2	1.39 ± 0.02
		SM	1189.4 ± 13.4	889.0 ± 12.2	1.35 ± 0.02
	Phytase	-	1193.2 ± 13.4	877.0 ± 12.2	1.37 ± 0.02
		+	1187.9 ± 12.8	867.0 ± 12.2	1.38± 0.02
Finisher phase	Feed	C	1691.3 ± 32.4	1017.2 ± 18.0^{b}	1.65 ± 0.04^{a}
		SM	1704.6 ± 36.2	1158.6 ± 18.0^{a}	1.48 ± 0.04^{b}
	Phytase	-	1675.0 ± 32.4	1071.7 ± 18.0	1.57 ± 0.04
		+	1721.0 ± 36.2	1104.1 ± 18.0	1.57 ± 0.04
Overall mean (d 0–36)	Feed	C	2883.1 ± 39.4	1872.2 ± 26.7^{b}	1.55 ± 0.24^{a}
		SM	2888.2 ± 41.3	2047.5 ± 26.7^{a}	1.43 ± 0.25^{b}
	Phytase	-	2869.5 ± 39.4	1948.7 ± 26.7	1.49 ± 0.24
		+	2901.9 ± 41.3	1971.1 ± 26.7	1.49 ± 0.25
P-values		SEM	8.7	8.9	0.01
	Grower phase	Feed	0.897	0.064	0.099
		Phytase	0.778	0.571	0.732
		F x P	0.724	0.667	0.310
		SEM	23.7	20.0	0.03
	Finisher phase	Feed	0.788	<0.001	0.004
		Phytase	0.356	0.219	1.00
		F x P	0.253	0.053	0.519
		SEM	27.4	26.2	0.02
	Overall mean	Feed	0.929	<0.001	0.002
		Phytase	0.577	0.559	0.885
		F x P	0.314	0.129	0.418

FI—feed intake, BW—body weight, BWG—body weight gain, FCR— feed conversion ratio, F x P—feed and Phytase interaction effect, − = without extra phytase supplementation; + = with extra phytase supplementation, d—days

In the study by (Lee et al., 2017) using a corn–soybean-based diet, the beneficial effects of phytase superdosing (1,500 FTU/kg) on feed intake and body weight

gain were evident at day 42 only in the low-phosphorus (P) diet group. Although phytase superdosing reduced FCR at all P levels ($P < 0.05$), the effect was more pronounced in birds fed the low-P diet. However, in this study, feeding SM to growers and providing enzyme supplementation throughout the experiment did not produce any differences in performance traits, and no interaction effect was observed between the enzyme supplement and the SM both on day 24 and day 36.

On day 36 SM at 30% inclusion level improved BWG and FCR. The reason could be, as broilers mature, their ability to utilize dietary fibre improves due to enhanced digestive enzyme production and development of a more robust gut microbiome. Furthermore, older birds are better able to tolerate the anti–nutritional factors present in SM, enabling them to maintain efficient nutrient utilization despite the higher fibre content (Munawar et al., 2025).

To conclude findings of the current study indicated that SM can be safely included in the broiler-grower diet up to 20%, and in the broiler-finisher diets up to 30% without any negative effects on production traits.

3.2.2. Dietary treatment effects on nutrient digestibility, nitrogen retention, and dietary metabolizable energy content

Inclusion of SM in broiler diet and phytase supplementation enhanced faecal nutrient digestibility, metabolizable energy and N-retention of dietary treatments (Table 16). Sunflower meal significantly improved the absorption of crude fat, and increased AMEn, however, affected negatively starch digestibility. The results suggest a negative correlation between the fibre intake and starch digestibility, which could be the results of the well-known diluting effects of fibre, which hinders the efficiency of α-amylase. As a result, HFSM in both experiment 1 and experiment 2 negatively affected starch digestibility. An important finding is that using extra phytase supplementation increased starch digestibility and all the measured parameters. Ravindran et al., (2002) reported phytase hydrolyses phytate, preventing its interaction with other nutrients, which may improve starch digestibility. It may also reduce saponification reactions between lipids and minerals complexed with the phytate molecule, thereby increasing energy utilization. In the study by (Fernandes et al., 2019a) increasing phytase levels between 1,000 and 1,500 FTU provided benefits in digestibility and metabolizable energy (AME and AMEn) coefficients when a maize–soybean meal-based diet was fed This result is in line with our findings that extra phytase increased AMEn and starch digestibility compared with the corn-wheat-soybean based control diet.

Table 16. Effect of dietary treatments on faecal nutrient digestibility and nitrogen retention

Feed	Phytase	Fat (%)	Starch (%)	AMEn (KJ/g)	N-retention (%)
C	-	90.2	86.9	13.5	80.1
	+	92.3	88.7	13.6	81.7
SM	-	94.3	84.3	14.1	79.7
	+	95.1	87.6	14.4	80.6
Feed	C	91.2^{b}	87.8^{a}	13.5^{b}	80.9
	SM	94.7^{a}	86.0^{b}	14.2^{a}	80.2
Phytase	-	92.2^{b}	85.6^{b}	13.8^{b}	79.9^{b}
	+	93.7^{a}	88.2^{a}	14.0^{a}	81.2^{a}
	SEM	0.36	0.33	0.07	0.30
P-value	Feed	<0.001	<0.001	<0.001	0.177
	Phytase	0.001	<0.001	0.019	0.031
	F x P	0.003	0.014	0.262	0.555

C—control, SM—sunflower meal, F x P—feed and Phytase interaction, +—plus phytase, - without phytase, AMEn—apparent metabolizable energy nitrogen corrected, − = without extra phytase supplementation; + = with extra phytase supplementation, ^{a,b} means with different superscripts in the same column are significantly different ($p<0.05$).

Opposite to starch, SM increased fat digestibility. The opposite trend in fat digestibility could be the fibre did not significantly disturb the micelle formation in the small intestine. Importantly, enzyme supplement improved faecal nutrient digestibility of all nutrients including N-retention. It was reported that the problem of high fibre contents in SM could be solved using NSPase enzyme which can spare some energy and protein to broilers (Bilal et al., 2017).

3.2.3. Effects of dietary treatment on ileal amino acid digestibility

The ileal amino acid digestibility of the experimental diets is presented in Table 17. The digestibility of AAs ranged between 60% (threonine) and 85% (methionine or arginine). Feeding the SM containing diet did not modify AA digestibility; however, phytase supplementation improved the digestibility of ARG, LYS, MET, VAL, and TYR. Leucine was the only AA negatively affected by phytase supplementation.

Table 17. Digestibility of essential amino acids as affected by dietary treatments (%)

Feed	Phytase	ARG	HIS	ILE	LEU	LYS	MET	PHE	THR	VAL	CYS	TYR
C	-	79.1	77.2	78.0	84.6	70.0	82.7	82.3	60.4	75.6	75.6	70.7
	+	83.7	78.0	80.0	83.4	74.2	84.8	82.9	68.2	77.7	73.5	78.6
SM	-	79.3	76.1	80.1	84.5	69.4	84.1	82.7	58.9	75.3	73.6	70.0
	+	85.1	77.9	80.2	81.7	74.1	85.0	82.5	66.8	78.4	74.1	78.9
Feed	C	81.4	77.6	79.0	84.0	72.0	83.7	82.6	64.3	76.7	74.5	74.7
	SM	82.2	77.0	80.1	83.1	71.7	84.6	82.6	62.8	76.8	73.8	74.5
Phytase	-	79.2 ^{b}	76.6	79.0	84.6^{a}	69.7^{b}	83.4^{b}	82.5	59.6^{b}	75.5^{b}	74.6	70.4^{b}
	+	84.5 ^{a}	77.9	80.0	82.5^{b}	74.1^{a}	84.9^{a}	82.7	67.5^{a}	78.0^{a}	73.8	78.8^{a}
	SEM	0.55	0.47	0.43	0.36	0.66	0.31	0.34	0.91	0.47	0.46	0.93
P-value	Feed	0.161	0.542	0.189	0.200	0.751	0.133	0.974	0.224	0.826	0.447	0.862
	Phytase	<0.001	0.176	0.220	0.003	<0.001	0.008	0.745	<0.001	0.006	0.425	<0.001
	F x P	0.321	0.615	0.265	0.214	0.870	0.262	0.617	0.959	0.593	0.177	0.703

C—control, SM—sunflower meal, F x P—feed and Phytase interaction, ARG—arginine, HIS—histidine, ILE—isoleucine, LEU—leucine, LYS—lysine, MET—methionine, PHE—Phenylalanine, THR—threonine, VAL—valine, CYS—cystine, TYR—tyrosine, − = without extra phytase supplementation; + = with extra phytase supplementation, ^{a,b} means with different superscripts in the same column are significantly different ($p < 0.05$).

3.2.4. Dietary treatment effects on length of the small intestine and relative organ weight

Sunflower meal and the extra phytase supplementation failed to modify the length of the duodenum, jejunum, and the length of the whole intestine ($p>0.05$), except for ileum, which was significantly shorter when SM was fed ($p=0.018$). No feed and intestinal part interaction effects were observed.

The inclusion of SM into the diets decreased the relative weight of liver, heart and ceca, but increased gizzard weight. Phytase supplementation increased the weight of the bursa of Fabricius (Table 18). The effect of including SM at 15%, 20% and 25% with and without NSPase enzyme supplementation was evaluated. The results revealed that relative gizzard and intestinal weights increased linearly with SM inclusion levels, whereas the relative liver and heart weights, and intestinal length were similar across treatments. Additionally, NSPase supplementation failed to affect the relative weight of the liver, heart, gizzard and the intestine (Bilal et al., 2017). Their results agree with our findings, that enzyme increased the weight of the Bursa fabricus, and SM increased gizzard weight.

Table 18. Dietary treatment effects on the relative organ weight (%)

Feed	Phytase	Gizzard	Liver	Heart	Spleen	Burca of Fabricus	Ceca
C	-	1.20	1.80	0.54	0.11	0.07	0.83
	+	1.22	1.75	0.50	0.09	0.14	0.66
SM	-	1.33	1.68	0.47	0.11	0.07	0.64
	+	1.47	1.67	0.50	0.09	0.10	0.55
Feed	C	1.21 [b]	1.78 [a]	0.52 [a]	0.10	0.10	0.75 [a]
	SM	1.41 [a]	1.68 [b]	0.49 [b]	0.10	0.09	0.61 [b]
Phytase	-	1.27	1.74	0.51	0.11	0.07 [b]	0.74
	+	1.34	1.70	0.50	0.09	0.12 [a]	0.60
	SEM	0.03	0.02	0.01	0.01	0.01	0.04
P-value	Feed	0.005	0.042	0.032	0.894	0.328	0.046
	Phytase	0.224	0.490	0.767	0.061	0.004	0.73
	F x P	0.339	0.704	0.072	0.586	0.169	0.601

C—control, SM—sunflower meal, F x P—feed and Phytase interaction, − = without extra phytase supplementation; + = with extra phytase supplementation (1500 FTU/kg), [a,b] means with different superscripts in the same column are significantly different ($p<0.05$)

3.2.5. Dietary treatment effects on gut pH and jejunum histomorphology

Compared with the control, animals fed SM showed a significantly higher duodenal and jejunal pH, probably due to higher alkaline pancreatic juice that buffers the acidic digesta from the gizzard, whereas caecal pH remained unaffected. Phytase supplementation failed to affect the gut pH (Table 19).

Table 19. Dietary treatments effects on the pH of gut content

Feed	Phytase	Duodenal pH	Jejunal pH	Caecal pH
C	-	6.38	6.24	7.68
	+	6.44	6.26	7.74
SM	-	6.51	6.42	8.07
	+	6.59	6.57	7.70
Feed	C	6.41[b]	6.25[b]	7.71
	SM	6.55[a]	6.50[a]	7.89
Phytase	-	6.45	6.33	7.87
	+	6.51	6.42	7.71
	SEM	0.03	0.04	0.07
P-value	Feed	0.005	<0.001	0.216
	Phytase	0.184	0.188	0.267
	F x P	0.814	0.308	0.135

C—control, SM—sunflower meal, F x P—feed and Phytase interaction, − = without extra phytase supplementation; + = with extra phytase supplementation, [a,b] means with different superscripts in the same column are significantly different ($p<0.05$).

Feeding SM reduced VH and MLT but increased VW. Phytase supplementation reduced VH, VW, VH/CD, and VSA.

Table 20. Dietary treatment effects on the jejunal histomorphology

Feed	**Phytase (P)**	VH (µm)	CD (µm)	VW (µm)	MLT (µm)	VH/CD	VSA (mm^2)
C	-	884.0	98.5	192.0	179.7	9.1	0.54
	+	780.8	97.0	169.2	187.1	8.1	0.44
SM	-	800.9	98.2	213.7	175.2	8.4	0.56
	+	738.7	94.8	195.2	152.9	7.8	0.46
Feed	C	832.4^{a}	97.8	180.6^{b}	183.5^{a}	8.7	0.49
	SM	769.8^{b}	96.5	204.4^{a}	164.1^{b}	8.1	0.51
Phytase	-	842.4^{a}	98.4	202.8^{a}	177.5	8.8^{a}	0.55^{a}
	+	759.7^{b}	95.9	182.2^{b}	170.1	8.0^{b}	0.45^{b}
	SEM	15.9	2.1	4.3	3.3	0.21	0.17
P-value	Feed	0.048	0.760	0.005	0.004	0.175	0.564
	P	0.009	0.562	0.014	0.263	0.048	0.003
	F x P	0.517	0.826	0.798	0.026	0.744	0.934

C—control, SM—sunflower meal, F x P—feed and Phytase interaction, CD—crypt depth, VW—villus width, MLT—muscle layer thickness, VH/CD— villus height to crypt ratio, VSA—villus surface area, − = without extra phytase supplementation; + = with extra phytase supplementation, ^{a,b} means with different superscripts in the same column are significantly different ($p<0.05$).

Sunflower cake, with and without enzyme complex, was fed to broilers from day 22 to day 42 at inclusion levels 0%, 5%, 10%,15%, and 20%. Sunflower cake at 15% and 20% decreased VH and the VH/CD ratio in the duodenum, jejunum and ileum, but increased CD in the jejunum (Berwanger et al., 2017). Their findings for VH were consistent with the current study.

3.2.6. Dietary treatment effects on the caecal SCFA contents

The dominant SCFA was acetic acid, followed by butyric and propionic acid (Table 21). Feeding SM did not affect SCFA contents, except for isovaleric acid, a similar result with experiment 1. Interestingly, extra phytase supplementation increased the amounts of acetic acid, propionic acid and butyric acid.

In chicken cecum, numerous bacterial families within the order of Clostridiales (*Lachnospiraceae, Ruminococcaceae*, and *Veillonellaceae*) are non-pathogenic and produce SCFA, including lactate, propionate, and butyrate (Rinttilä and Apajalahti, 2013). In our first experiment the inclusion of high fibre SM and low fibre SM at 20% and 30% levels did not improve the SCFA concentrations which supports the current findings, except for isovaleric acid.

SCFA production is influenced by the availability of fermentable substrates, and increasing dietary fibre does not necessarily lead to higher SCFAs production. In the gastrointestinal tract of poultry Acetate is the predominant SCFA, followed by propionate or butyrate depending on the diet and the specific gut region. Among these, butyrate has a beneficial effect on intestinal cell nutrition and supress pathogenic bacteria in the gut (Singh and Kim, 2021). Similarly, the dominant SCFAs in the current study were acetic acid followed by propionate and butyrate. Interestingly, enzyme supplementation improved three of them. Based on the results of this experiment, extra phytase increases the SCFA contents of SM containing diets.

Table 21. The effect of dietary treatment on the SCFA contents **(µmol/g)**

Feed	Phytase	Acetic Acid	Propionic Acid	Iso-butyric Acid	Butyric Acid	Isovaleric Acid	Valeric Acid
C	-	1.98	0.46	0.06	0.49	0.07	0.10
	+	2.19	0.59	0.06	0.71	0.07	0.10
SM	-	2.00	0.49	0.06	0.49	0.09	0.10
	+	2.23	0.60	0.08	0.67	0.10	0.10
Feed	C	2.08	0.53	0.06	0.60	0.07 ^{b}	0.10
	SM	2.11	0.55	0.07	0.58	0.09 ^{a}	0.09
Phytase	-	1.99 ^{b}	0.47 ^{b}	0.06	0.49 ^{b}	0.08	0.10
	+	2.21 ^{a}	0.60 ^{a}	0.07	0.69 ^{a}	0.08	0.10
	SEM	0.03	0.01	0.004	0.02	0.006	0.005
P-value	Feed	0.418	0.305	0.354	0.540	0.027	0.650
	Phytase	<0.001	<0.001	0.062	<0.001	0.681	0.957
	F x P	0.783	0.527	0.270	0.568	0.886	0.646

C—control, SM—sunflower meal, F x P—feed and Phytase interaction, − = without extra phytase supplementation; + = with extra phytase supplementation, ^{a,b} means with different superscripts in the same column are significantly different ($p<0.05$).

3.2.6. Gut microbiota

In this study, from all 62 samples, a total of 1,695,668 good-quality 16S rRNA reads were available for analysis after quality filtering. The average counts of sequence were 31,733 in the ceacal chyme (CC) (min: 1,148; max: 62,276). These sequences were assigned to 1072 OTUs at 97% similarity using the open approach.

3.2.6.1 Diversity indices

Feeding the SM containing diets significantly increased the microbial richness in the cecum, as indicated by the Observed ($p = 0.042$) and Chao1 ($p = 0.043$) indices (Figure 2.). However, no significant differences were observed in the Shannon and Simpson diversity indices ($p > 0.05$), suggesting that while the total number of detected taxa increased, the overall evenness and structural complexity of the microbial community remained stable across treatments.

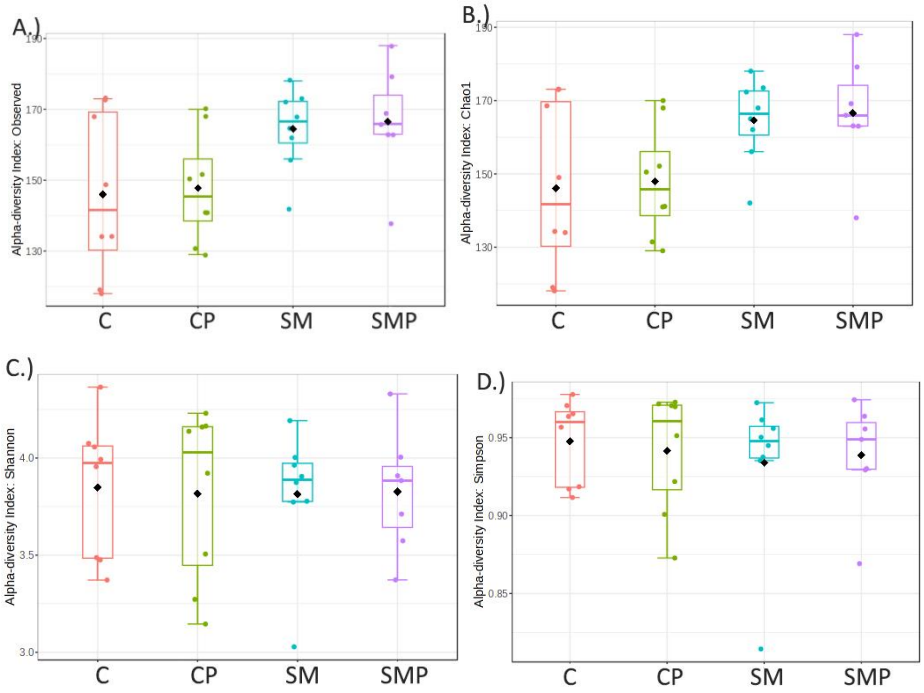

Figure 2.Alpha diversity indices of the caecal microbiota

A: Observed p-value: 0.041978; [ANOVA] F-value: 3.1311; **B:** Chao 1: p-value: 0.043016; [ANOVA] F-value: 3.1071; **C:** Shannon: p-value: 0.99787; [ANOVA] F-value: 0.013228; **D:** Simpson: p-value: 0.91375; [ANOVA] F-value: 0.1729

Beta diversity analysis revealed a significant shift in the microbial community structure (Figure 3.). The highly significant difference in Unweighted UniFrac distance ($p = 0.001$, $R^2 = 0.18$) indicates that SFM supplementation introduced distinct bacterial lineages that were absent in the control group. This separation was further supported by the Jensen-Shannon Divergence ($p = 0.043$).

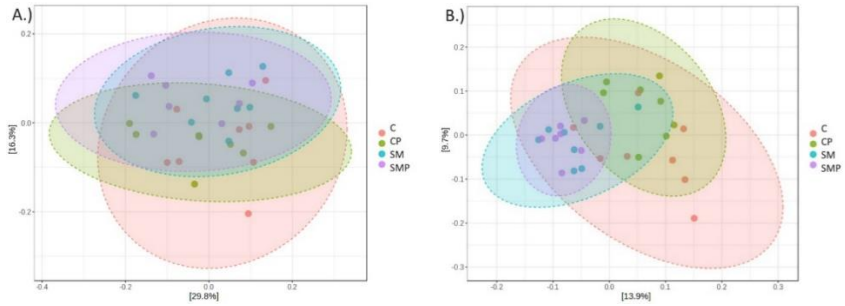

Figure3. Beta diversity indices of the caecal microbiota

A: Pcoa Jensen-Shannon Divergence, Permanova (F-value: 1.6785; R-squared: 0.15719; p-value: 0.043); B: Pcoa Unweighted Unifrac Distance, Permanova (F-value: 2.0284; R-squared: 0.18392; p-value: 0.001)

3.2.6.2 Relative abundance of bacterial taxa

Firmicutes was the major dominant phylum in all four treatments. The dietary treatments influenced the abundance of two minor phyla. Both Tenericutes (p=0.004) and Actinobactera (p=0.001) were significantly higher under the influence of SM treatment. At the class level the relative abundance of the Coriobacteriia (C:0.12% SFM:0.91%; p=0.001) and the Mollicutes (C:0.67% SFM:1.47%; p=0.006) were significant higher in the treatment SM than in the C group. Likewise, at the order level the Coriobacteriales and Mollicutes_RF39 were also significantly higher in the SM treatment group. The dominant families were the two-butyrate producing family, Ruminococcaceae and Lachnospiraceae. Significant differences were found only in the case of some minor families which were higher in SM groups (Not assigned (P= 0.03), Eggerthellaceae (P= 0.002), Family_XIII (P< 0.001)).

No significant treatment effects were found in the bacterial genera above 1% abundance. However, the abundance of some minor genera was affected. Among them *Lachnospiraceae_UCG_010*, *Ruminococcaceae_UCG_007*, *Ruminococcace_UCG_009,* *Ruminiclostridium_9* and *Ruminococcaceae_V9D20*13_group are well known butyrate producing families. Their abundance increased significantly in the SM groups.

Our results support the hypothesis that substrate availability specifically the increase in non-starch polysaccharides (NSP) and phenolic compounds is the primary driver of microbial shifts, while phytase supplementation exerted only a secondary, modulating effect. The alpha and beta diversity indices showed that the diverse fibre content of SM increase the number of identifiable bacterial

groups and at certain extent also the structure of bacteria. It could be considered as a positive effect.

Genera that significantly decreased in the SM and SMP groups *(Blautia, Eubacterium_hallii_*group*, Lachnospiraceae_UCG-001)* are characterized by saccharolytic and cross-feeding metabolic pathway. Eubacterium hallii and Blautia (member of Lachnospiraceae family) are key players in the gut metabolic network, specializing in the conversion of lactate and acetate to butyrate. The decline of these taxa suggests a reduced influx of easily fermentable sugars and starch to the cecum in birds fed SM. *Blautia* species are highly efficient at utilizing simple carbohydrates but are often outcompeted in environments dominated by complex, lignified fibres ((Engels et al., 2016; Liu et al., 2021).

At the phylum level, the significant increase in Actinobacteria and Tenericutes in the SM-fed groups validates the metabolic shift towards complex substrate utilization. Actinobacteria produce enzymes that decompose polysaccharides or phenolic compounds in dead plant biomass, like Chlorogenic acid (CGA). The bacterial genera that increased in frequency in the SM and SMP groups had a total increase of 2.77 and 2.83%. The genus that belongs to the Actinobacteria phylum among the listed, significantly variable, is Gordonibacter. Members of this genus are known to be able to metabolize dietary polyphenols (e.g. ellagitannins and chlorogenic acid – which are abundant in sunflower) into bioactive metabolites, such as urolithins (Selma et al., 2014). This suggests that SM may enhance the antioxidant potential of the gut through microbial biotransformation.

Sunflower meal-based diets led to increase the abundance of some taxa specialized in the degradation of complex plant cell wall polysaccharides. The significant increase in Ruminococcaceae (UCG-007, UCG-009, V9D2013) and Ruminiclostridium 9 suggests adaptation to the higher crude fiber and hemicellulose content of sunflower meal. These genera possess an extensive range of carbohydrate-active enzymes, such as cellulases and xylanases, which are required for the anaerobic fermentation of plant fibers (Biddle et al., 2013; Jensen et al., 2021; You et al., 2023).

4. CONCLUSIONS AND RECOMMENDATIONS

According to the results of the experiments, it can be concluded, that broiler chickens have higher fibre tolerance than can be found in the literature. Feeding high-fibre and low-fibre SM at 20 and 30% inclusion rates did not result depression in the production parameters. It seems the chickens have fibre "hunger" in the finisher phase, and this fibre rich diet can improve growth rate without affecting FCR. The reason for these positive results could be the gizzard stimulation of the dietary fibre, that has several positive consequences. This theory is supported by the increased gizzard weight and the improved digestibility of fats and amino acids when SM was fed. The reason why SM decreased the digestibility of starch could be, that the structural fibre of SM probably increases the passage rate in the small intestine and starch digestion is more sensitive towards this change.

It is an important finding, that the AA digestibility modifying effect of SM is amino acid dependent. The reason could be the extended gizzard – proventriculus retention of the ingesta, the increased hydrochloric acid secretion, the lower pH and the stimulation of the pancreatic protease enzyme secretions. The differences in the efficiency of pepsin and the pancreatic proteases can cause amino acid specific release and absorption from the small intestine. Consequently, the digestibility of leucin was decreased when SM was fed. It was the only amino acid affected negatively. The reason could be that SM increase endogenous protein losses (mucine, enzymes, epithelial erosion) in the small intestine and these proteins are rich in leucine.

Probably we have evaluated the SM effects on the AA digestibility from the different small intestine parts for the first time. From this aspect the low- and high-fibre SM worked in a different way. The LFSM effect was more pronounced in the ileum and increased the digestion of most of the amino acids.

In the frame of SID amino acid digestibility determination, the samples were taken from the whole ileum or from its distal part, since some amino acid digestion still can happen in the proximal ileum. According to the present results, except for two amino acids (TYR and VAL) no significant differences exist in the AA digestibility between the two parts of ileum. The proximal ileal digestibility values are 98-99% of the distal values. On the other hand, the digestion dynamics of the amino acids is different in the jejunum. Higher absorption rate is true for ARG, PHE, and LEU, but lower disappearance for CYS, ILE, THR and LYS. Not only the AA digestibility but its dynamics was also affected by the SM treatments.

The speed of MET and ILE disappearance increased if SM containing diets were fed.

Feeding sunflower meal affects also the environmental aspects of animal production. It decreased the urinary and total nitrogen excretion of birds, which is important from ammonia emission mitigation point of view. The fibre effect on the urinary N excretion is well known for pigs, when the bacterial fermentation increase in the large intestine and bacteria can convert the ammonia to bacterial protein. This decreases the urinary N excretion, which is the main source of ammonia emission. This mechanism is limited in birds, due to the anatomic differences, but according to this fibre effect it works also in the chicken.

In our previous work, the extra phytase supplementation of laying hen diets improved nutrient digestibility. The same results were found also in this case with broiler chickens. The mechanism behind the positive effects of extra phytase is the more complex breakdown of the phytate bounds, including also the protein and starch matrixes. But it is also important finding from practical point of view, that not all the improvements on the digestion can be converted to production traits. According to our caecal SCFA results extra phytase can also make free some fermentable fibre fractions, that can get into the caeca of birds.

Sunflower meal contains mainly structural fibre, but also several fermentable oligosaccharides. Feeding SM do not cause big differences in the caecal microbiota, but significant differences have been found in some minor taxonomic groups. The higher alpha diversity indices and the significant differences in the beta diversities suggest, that feeding SM results more divers and stable bacteriota composition. It is positive from gut health point of view.

In summary, our findings indicated that, in isonitrogenous and isocaloric diets, HFSM and LFSM can be used as a substitute for soybean meal in broiler diets at levels up to up to 20% (grower) and 30% (finisher).

7. NEW SCIENTIFIC RESULTS

1. Feeding high-fibre sunflower meal (CF: 16–17%) with broiler chickens at 30% resulted in a significantly higher body weight gain in the finisher phase compared with the control, corn-soybean-wheat based diet.
2. Feeding sunflower meal at 20 and 30% inclusion rates resulted in significant increase in fat digestibility, in the AMEn content of the diets, but decreased starch digestion. The differences were age, fibre and inclusion rate dependent.
3. Sunflower meal containing diets increased the apparent digestibility of many essential amino acids compared with the control diet. The highest improvements were found in the case of ARG, HIS, ILE, LYS, MET, THR, VAL, TYR. The digestibility values were affected by the age of chickens, the intestinal segments, the fibre content and the inclusion rate of sunflower meal.
4. On day 41, feeding SM increased the disappearance rate of MET and ILE from the distal jejunum.
5. On day 27, the inclusion of HFSM and LFSM at 30% level, significantly decreased the uric acid-N and total N excretion of chickens, which is a positive result from ammonia emission point of view.
6. Using extra phytase supplementation (1500 FTU/kg) of SM containing diets do not modify the production traits but significantly increases the digestibility of fat, starch, amino acids (ARG, LYS, MET, THR, VAL, TYR), the N-retention of animals, the AMEn content of diets, and the short chain fatty acid content of the caeca.
7. Feeding SM at 30% inclusion rate, increased the alpha diversity and the abundance of some butyrate producing genera *(Ruminococcaceae, Lachnospiraceae*) in the caecal content of the 42-day old broiler chickens.

8. PUBLICATIONS IN THE RESEARCH FIELD

8.1. Articles published in peer-reviewed and impact factor journals

1. **Tewelde, K.G.,** Kiss, B., Csiszér, T., Pál, L., Such, N., Bartos, Á., Dublecz, K., 2026a. Feeding Low- and High-Fibre Sunflower Meal to Broiler Chickens—Effects of Inclusion Rate and Age of Birds on the Production Traits, Carcass Composition, Nutrient Digestibility, Gut Viscosity, and Caecal Short-Chain Fatty Acid Content. Animals 16, 162. https://doi.org/10.3390/ani16020162 (Q1; IF:3.2; CiteScore:5.2)
2. Such, N., Mezőlaki, Á., **Tewelde, K.G**., Pál, L., Horváth, B., Poór, J., Dublecz, K., 2024. Feeding sunflower meal with pullets and laying hens even at a 30% inclusion rate does not impair the ileal digestibility of most amino acids. Front. Vet. Sci. 11. Pp. 1-11. https://doi.org/10.3389/fvets.2024.1347374 (Q1; IF:3.3; CiteScore:5.1)
3. Mezőlaki Á., Such, N., Wágner, L., Rawash, M. A., **Tewelde, K. G**., Pál, L., Poór, J., Dublecz, K. (2023): Evaluation the nutrient composition of extracted sunflower meal samples, determined with wet chemistry and near infrared spectroscopy. Journal of Central European Agriculture, 2023, 24(3), p.613-623. https://doi.org/10.5513/JCEA01/24.3.3812 (Q3; IF: 0.7; CiteScore:1.1)

8.2. Papers published in full in a conference publication

1. **Tewelde, K.G.,** Such, N., Kiss, B., Pál, L., Dublecz, K., 2026b. Potentials and constraints of sunflower meal as an alternative protein source for broiler chickens, in: 24. BOKU-Symposium Tierernährung 2026. Boku, Viena, pp. 74–76.
2. Such, N., Mezőlaki, A., Kiss, B., Pál, L. Rawash, M. A., **Tewelde, K. G**., Dublecz, K. (2024): Effect of feeding extracted sunflower meal-based diets, with and without NSP degrading enzyme, on the viscosity of the jejunal and ileal intestinal content of pullets and laying hens. 22. Boku- Symposium Tierernährung, 29. Februar, Viena, proceeding book, pp.184- 187.

8.3. List of abstracts and posters presented in a conference

1. **K. Tewelde**, N. Such, T. Csiszér, L. Pál , B. Kiss , L. Wágner, K. Dublecz. (2025). Effects feeding low and high fibre sunflower meal on the production traits of broiler chickens. In 24th European Symposium on Poultry Nutrition (ESPN). Maastricht, Netherlands, 2025. June 23–26. (p-270). https://www.espn2025.eu/wp-content/uploads/2025/06/ESPN-2025-Abstractbook-final.pdf

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7. APPENDICES

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