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

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LIST OF ABBREVIATIONS

OECD	Organization for economic co-operation and development
FAO	Food and agriculture organization
EC	European commission
EU	European union
WITS	World integrated trade solution
SM	Sunflower meal
DM	Dry matter
CF	Crude fibre
EE	Ether extract
NDF	Neutral detergent fibre
ADF	Acid detergent fibre
IDF	Insoluble dietary fibre
SDFP	Soluble dietary fibre precipitable
GE	Gross energy
NFE	Nitrogen free extract
CP	Crude protein
AA	Amino acid
EAA	Essential amino acid
SID	Standard ileal digestibility
AID	Apparent ileal digestibility
AAD	Amino acid digestibility
NSPs	Non-starch polysaccharides
GIT	Gastrointestinal tract
FI	Feed intake
AFI	Average feed intake
LW	Live weight
WG	Weight gain
AWG	Average weight gain

BWG	Body weight gain
FCR	Feed conversion ratio
ME	Metabolizable energy
AME	Apparent metabolizable energy
AMEn	Apparent metabolizable energy corrected for nitrogen
TME	True metabolizable energy
SCFAs	Short chain fatty acids
LFSM	Low-fibre sunflower meal
HFSM	High fibre sunflower meal
CGM	Corn gluten meal
DDGS	Dried distillers' grains
TDN	Total digestible nutrients
ARG	Arginine
HIS	Histidine
ILE	Isoleucine
LEU	Leucine
LYS	Lysine
MET	Methionine
PHE	Phenylalanine
THR	Threonine
VAL	Valine
CYS	Cystine
TYR	Tyrosine
ALA	Alanine
ASP	Aspartic acid
GLU	Glutamic acid
GLY	Glycine
PRO	Proline
TRY	Tryptophan
SER	Serine

IDE	Ileal digestible energy
SBM	Soybean meal
MAB	Hungarian Accreditation Committee
SCFA	Short chain fatty acid
ANOVA	Analysis of variance
PERMANOVA	Permutational analysis of variance
PCoA	Principal coordinate analysis
OUT	Operational taxonomic unit
MCP	Monocalcium phosphate
FTU	Phytase titration unit/ phytase unit
FYT	Phytase unit
SMP	Sunflower meal with extra phytase
CP	Control diet with extra phytase
QIIME	Quantitative insights into microbial ecology
BXU	Birchwood xylan units
VH	Villus height
CD	Crypt depth
VW	Villus width
MLT	Muscle layer thickness
VSA	Villus surface area
CC	Caecal chyme

1. INTRODUCTION

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, with increasing demand for poultry in the Middle East, Africa and Asia. Poultry production in the EU is also estimated to increase slightly, driven by shifts in consumption and expanding export opportunities. This will result in an increase of about 965,000 tonnes (+ 0.7% annually) between 2025 and 2035. However, stricter environmental regulations and the transition to more sustainable production systems may limit the growth to certain regions within the EU (EC, 2025).

Climate change impacts agricultural yields through global warming, extreme weather events, and changes in precipitation patterns. This leads to reduced yield, lower affordability, and price instability. It also affects global trade, especially for imported crops and animal feed, which in turn affects food security (Jensen and Hourdin, 2025). This indicates that food and feed market instability is increasing, mainly due to climate change, which reduces crop production prediction (OECD/FAO, 2025).

In the case of broiler chickens, feed cost accounts for about 70% of the total production cost (Adaszyńska-Skwirzyńska et al., 2025; Alhotan, 2021; Desai et al., 2018) . 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 in high and efficient yielding animal production is soybean meal. It has to be imported from the American continent to various parts of the world that resulted in increased price due to long transportation cost and other external fluctuations (Shi et al., 2012), as a result, feed manufacturing industries, poultry farmers, and researchers are looking for locally available, cheaper alternative protein sources in order to replace maize and soybean in ration formulations. This is a better approach to overcome increasing feed cost. As a result, the use of industrial by-products and locally grown protein crops as poultry feeds are expected to increase in the future (de Visser et al., 2014). However, the feed manufacturing companies could not find an alternative that could fully replace soybean meal. Due to this reason, many countries, including EU member countries, are highly dependent on imported protein sources, mainly soybean meal (WITS, 2024).

Peng et al. (2025) highlighted that global soybean trade has rapidly expanded, the trade dynamics are driven by climate, and the expansion has caused deforestation, biodiversity loss, and food security challenges. In addition, it was found that one third of 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 (Aziz and Rashid, 2023; Dotas et al., 2025).

The increase in demand for soybean in the global market has gained an increasing importance, in Brazil. This leads to the expansion of cultivable land to increase the production of soybean through clearing forests. Its expansion is a big threat to climate change resulting from deforestation (Lathuillière et al., 2017). Therefore, the reliance on a single protein source (soybean) may cause availability, production cost, and environmental problems, which are currently forcing researchers and nutrition experts to search for alternatives (Al-molah et al., 2023; Shi et al., 2012). Possible alternatives are legume seeds, extracted meals, dried distilled grains with solubles, meat meals, fish meal, insect meal, and seaweeds (Tewelde et al., 2026a).

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 (Mbukwane et al., 2022). 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 (Berger et al., 2021). 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.

Sunflower is a widely cultivated, important oil crop that ranks third in oilseed and seed-meal production after soybean and rapeseed meal, as well as fourth among the vegetable oils such as palm, soybean, and rapeseed oils. This indicates that it is both competitive and abundantly available (Konyalı, 2017; Pilorgé, 2020; Rodríguez et al., 2005). In addition, the advantages of using SM as an alternative protein source include a lack of antinutritional factors, relatively low cost, richness in sulphur-containing amino acids, and rich sources of crude protein (CP) and essential amino acids (EAAs). Additionally, SM is high in calcium, phosphorus, and vitamin B-complex. The only limitations are its high fibre, lysine and threonine deficiencies, low metabolizable energy, and some polyphenolic compounds such as chlorogenic contents when compared with soybean meal (Aziz and Rashid, 2023; Chobanova, 2019; Mezőlaki et al., 2023) . This can be solved through a careful feed formulation (Mbukwane et al., 2022). The low energy and lysine levels can be compensated through fat and lysine supplementation (Mezőlaki et al., 2023).

According to different reported feed-analysis results, the dietary fibre of SM ranges from 10% to 26% (Attia et al., 2003; Li et al., 2018; Papanikou, 2020; Pereira and Adeola, 2016; Tewelde et al., 2026a)and its CP ranges from 29.5% to 44% (Attia et al., 2003; Tewelde et al., 2026a). The fibre and nutrient content vary according to the results of different degrees of dehulling, meal or cake production system, variety of sunflower and degree of oil extraction.

Cell wall of SM contains non-starch polysaccharides (NSPs), including β–glucans, xylans, arabans, pectins and oligosaccharides, which are known to increase digesta viscosity, reduce nutrient utilization, and consequently depress growth in chickens (Senkoylu and Dale, 1999). No result has been reported about the degradation of NSP in SM by chickens (Lannuzel et al., 2022).

Sunflower seed contains both structural and water-soluble fibre components. The insoluble fibre found in the hull can stimulate gizzard development and increase the digesta retention time in the upper part of the gastrointestinal tract (GIT). This enhancement in gizzard size and function may stimulate pancreatic enzyme secretion, thereby improving the digestibility of starch, lipids, and other nutrients in the upper GIT (Jha and Mishra, 2021). In contrast, the dominant water-soluble fibre fractions in sunflower, particularly β -glucans, increase digesta viscosity and impair nutrient absorption (Alagawany et al., 2017; Tejeda and Kim, 2021). This can be mitigated through the use of exogenous enzyme supplementation. The nutritional value of SM can be significantly affected by chemical, mechanical, and thermal processing methods during oil extraction from the seed. These treatments may result in anti-nutrients, complex protein structures, insufficient amino acids, and high fibre content, all of which can reduce the digestibility and limit utilization, particularly in younger broilers. Therefore, the use of NSP enzymes, along with careful management of inclusion rates, can help improve its digestibility and overall effectiveness as a feed ingredient (Ditta and King, 2017).

The use of exogenous enzymes improves the value of agro-industrial and agroforestry waste 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 complex materials, so enzyme treatment has become an effective strategy to increase productivity and animal performance (Ojha et al., 2019; Ravindran, 2013). Therefore, supplementation of compound NSP enzymes (β- mannanase, β-glucanase, xylanase, and cellulase) in the diet can minimize the negative effects of NSPs and improve the digestion and utilization rate of nutrients. However, results from exogenous enzyme supplementation in broiler diets are found inconsistent, with some studies showing improved performance , and others reported no improvement (Amerah et al., 2015; Ciurescu et al., 2019; de Oliveira et al., 2016; Desai et al., 2018; Mbukwane et al., 2022; Molale, 2020; Yaqoob et al., 2022)

In summary, most studies investigating SM supplementation employ multienzyme complexes, making it difficult to distinguish the individual effects of specific enzymes and leading to inconsistent results. Recent research approaches suggest that improving digestibility should not focus solely on NSP-degrading enzymes. In the case of soybean-based diets, phytase supersdosing supplementation was used effectively in some research, where the focus of its effectiveness is not only the higher availability of phosphorus, but an additional physiological effect of phytase superdosing (Fernandes et al., 2019). The main effect of phytase favours the availability of the nutrients that would be complexed to the phytic acid molecule. Therefore, resulted in improvement of animal performance. The reason for the improvement was that Phytic acid has a strong binding affinity to nutrients such as soluble proteins at low pH, amino acids, minerals, and starch (Yu et al., 2012). In case of SM, it is even more effective, because research has shown that the high phytate content (3 – 4% of dry matter, or 80 – 85% of phosphorus) of SM decreases the digestibility of nutrients and utilization of phosphorus in poultry (Krum, 2023). In a study high protein SM was added in to broilers diet at proportions of 0.05%, 0.10%, and 0.15% of the total phosphorus, replacing corn-starch with or without an enzyme phytase (500 U/kg). Their result showed that the enzyme supplemented SM-based-diet had improved apparent ileal digestibility (AID), BWG, feed efficiency, and bone traits (Kim et al., 2019). The addition of 0.2 g/kg microbial phytase had no effect on BWG, FI and FCR (Ciurescu et al., 2019). In a soybean-based diet, Poernama et al. (2021) concluded that their findings confirmed the efficacy of phytase and its synergistic effect with NSPase, as birds showed further improvements in growth performance following NSPase supplementation in the presence of phytase. 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.

Mbukwane et al. (2022) concluded that dehulled sunflower oilcake can be incorporated in finisher and post finisher diets up to 13.5% without negatively affecting the feed intake (FI), body weight gain (BWG), and feed conversion ratio (FCR). Several experiments were conducted to study the effects of feeding SM to broilers on production performance and carcass characteristics (Yaqoob et al., 2022), digesta viscosity (Amerah et al., 2015; Munawar et al., 2025; Such et al., 2024b), AME (Saleh et al., 2021), nutrient digestibility (Barua et al., 2021; Kadim et al., 2002; Ravindran et al., 1999; Such et al., 2024a), excreta-N composition (El-Deek et al., 2020), and nitrogen retention and energy utilisation (Harn et al., 2019; Strifler et al., 2023).

The inclusion of SM in the broiler diet mixed along with other low-protein diets using a 15% combination of corn gluten meal (2.5%), dried distillers' grain (7.5%), and SM (7.5%) compared with the control diet after formulating isonitrogenous and isocaloric diet failed to affect the total excreta-N composition (El-Deek et al., 2020). Other findings revealed that feeding low-protein diets or reducing the protein content of the broiler ration resulted in reduced N excretion (Harn et al., 2019; Strifler et al., 2023; Such et al., 2021). In addition, reducing dietary protein with a balanced amino acid supply does not affect dietary AMEn, it even improves nitrogen retention and energy utilisation (Harn et al., 2019; Strifler et al., 2023).

The above-mentioned characteristics of SM make it a better alternative to minimise the dependency on the traditional crops in chicken production so as to reduce production cost and the environmental impact of importing soybean meal far from Latin America.

2. OBJECTIVES

Examining the use of different levels of SM in broiler diet formulation on production performance, digestibility and environmental impact is currently a hot topic to determine its effect and the possible maximum inclusion rate in broiler diet. Though limited information is available on the maximum inclusion levels of SM in broiler diets, Such et al. (2024a) 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 in the diet of the animals mentioned. There is no research directly comparing low-fibre and high-fibre sunflower meal in broiler-grower and broiler-finisher phases on the caecal short chain fatty acids (SCFAs), digestibility of AMEn and excreta-N composition. As per the knowledge of the authors, there is also no research that includes low-fibre SM (LFSM) and high-fibre SM (HFSM) at up to 30% of the broiler diet.

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 acids, and digesta viscosity.
- To evaluate the ileal amino acid digestibility of broiler diets that contain high and low fiber SMs both at the grower and the finisher phases. Furthermore, compared the AA absorption in the distal part of the jejunum, the proximal part of the ileum and the distal part of the ileum during the finisher phase in order to get more understanding on how SM can affect the dynamics of AA absorption in the different parts of the small intestine.
- To investigate the effects of feeding sunflower meal and extra dietary supplementation of phytase to the diets on the production traits, nutrient digestibility, organ weight, gut content pH, histomorphology, intestinal length, and short chain fatty acid contents of hind gut chyme.

3. LITERATURE REVIEW

3.1. Sunflower and its origin

Sunflower is one of the four highly ranked oilseeds grown for edible oil such as soybean, rapeseed, and peanut. Traditionally sunflower oil has commanded a premium over soybean oil on the world markets (Putt, 1997). There are two types of sunflowers grown. The one with black seed colour and thin hull is the high oil seed variety ranging from 40 to 51% oil content. The second variety is the thick hulled mainly used in snack, confectionery, bakery, and birds' food market probably because of its low oil, 25%, content (Park et al., 1997). Sunflower (*Helianthus annuus*) is an annual or perennial plant used for manufacturing oil, oil by-products, and sunflower meal (Putt, 1997). Depending on the variety, sunflowers mature and produces seed in the range 80 to 120 days (Profundo, 2022).

Multiple domestication and breeding bottlenecks resulted in the modern single-cross hybrids of sunflowers (*Helianthus annuus L.*) (Tang and Knapp, 2003). The wild-growing annual species of sunflower are believed to be where the modern single hybrid Sunflower (*Helianthus annuus L.*) is originated from. This took a multiple domestication and breeding process. The annual sunflower *H. annuus* originated in eastern central north America and it is about 5000 years since sunflower was cultivated (Blackman et al., 2011; Gavrilova and Anisimova, 2017; Park and Burke, 2020; Senkoylu and Dale, 1999). Particularly, Blackman et al. (2011) reported that, based on multiple evolutionary important loci and neutral markers, eastern North America is the place of origin for the extant cultivated sunflower.

3.2. Sunflower seed, oil, and meal production

Generally, the oil content of sunflower seeds can be extracted by cold or hot-pressing process. The by-product of oil extraction with the hydraulic press is the cake (sunflower cake). If the oil seeds are pressed through a perforated cylinder with a narrowing hole, expellers are produced. The cold pressing is also followed by extraction with some fat diluent (Zoltan Peter, 2018), the use of solvents to further extract the oil resulted in solvent extracted SM.

Sunflower seed production increased from 48.87 million metric tons in 2021 to 55.98 million metric tons in 2024 in the world (USDA, 2025a, 2025b). Similarly, in EU the production increases from 9.39 (in 2023) to 10.08 million metric tons (in 2024) (USDA, 2025b), indicating an increase in SM production. As a result, SM production also increases from 20.28 to 23.24 million metric tons within five years since 2021/2022. In the world supply and distribution comparison sunflower rank 4th from the major vegetable oils, and 3rd from both the major oilseed and meal production after soybean and rapeseed from 2021 to April 2025. Importantly overall protein meal production increases by 10.95% from 2020 (350.08 million metric ton) until April 2025 (388.47 million metric tons) (USDA, 2025a). This implies an efficient and optimum utilization of seed meal as animal feed is imperative.

The producers of sunflower are the first consumers of the meal. Countries such as the EU, Ukraine, Russia, Argentina, Turkey, China, and the USA produce 90% of the sunflower meal and consume 81% of the world's meal production. Ukraine, Russia, and Argentina are the only net exporters. The EU, in spite of its high production, is also the first importer. Belarus, India, Morocco, and Israel are regular importers in significant quantities (Pilorgé, 2020). Ukraine is the top producer of sunflower seed since 2008/09 but in the marketing year of 2022/23, became the third leading producer after Russia and the EU (USDA., 2022a, 2022b).

Among the major protein meal seeds, sunflower meal ranks third after soybean meal and rape seed meal from 2008 to October 2022 with a worldwide production potential between 20.94 to 21.89 million metric tonnes per year and this rank is maintained until 2025 (USDA, 2025a; USDA., 2022a). These figures showed the economic significance of sunflower meal as broiler feed. However, the main limiting factor as chicken feed is its high fibre content. There is also a problem in its maximum inclusion rate in broiler diets due to the difference in variety, oil extraction methods and degree of dehulling applied. Therefore, the knowledge of meal production is very important in poultry nutrition point of view.

3.3. Economic aspects of feeding extracted sunflower meal

Sunflower meal is one of the cheapest and main dietary protein sources for animals. The benefits of sunflower meal over the other source of proteins are its high content of methionine and cysteine, comparatively low cost, and lack of antinutritional factors (Chobanova, 2019). Toasted full fat SM was included at 0%, 5%, 10%, 15% and 20% in the broiler diet. Broilers tolerate up to 5% level without affecting the performance and carcass yield and significantly reduced feed cost (Mohammed et al., 2020). The price of SM varies depending on region and type of SM. The EU average price in 2025 was 218.8 €/ton (ranging from 170.86 € to 312.37 €/ton) (TESEO, 2025).

Replacing 25 to 50% soybean meal with SM in the starter, finisher and overall phases (0 to 35 days) had no detrimental effects on feed intake, body weight gain, and gain to feed ratio. This implies that the dietary inclusion level of high protein SM up to 8. 6 to 17. 2% of the broiler diet was found a feasible strategy to reduce feed costs without affecting growth performance (Waititu et al., 2018b). Replacing soybean meal with SM, corn gluten meal (CGM) and dried distillers' grains (DDGS) at 15% improves the economics of broiler production and supports the sustainability of broiler production (El-Deek et al., 2020). Another study concluded that SM at 13% and 13.5% in the finisher and post finisher diets respectively, supplemented with enzymes result in substantial reduction in feed price while yield remain unaffected (Mbukwane et al., 2022). The effect of dietary inclusion of SM on the economic efficiency of broilers was investigated by Attia et al. (2016). Their findings revealed that the inclusion of SM at 0%, 2.5%, 7.5%, and 10% had no significant difference in their economic efficiency, however birds fed 10% SM had the highest (86.65%) and birds fed the control diet had the lowest (82.42%) economic efficiency.

Sunflower meal replacement at 10 and 5% record better profit per live weight when compared with the control and 15% inclusion levels. Sunflower meal at 5% and 10% levels records the highest profit margin but when supplemented with an enzyme protease the highest profit margin was recorded at 15% level (Lemme et al., 2004). Enzyme supplementation to a sunflower corn-based diet was found more feasible and economical to get maximum profit as the maximum net profit obtained from enzyme-supplemented diets was 11.3 times higher than the diet without enzymes (Khan et al., 2006).

The main aim of searching alternative protein sources such as SM is to replace soybean meal. The price of different sunflower meal products varies depending on its CP content and additionally included supplements to compensate the deficiency in SM. High protein SM is more expensive than low protein SM. However, high protein SM is still cheaper than soybean meal. Therefore, replacing soybean meal with low cellulose SM plus necessary supplements is still profitable (Nedelkov, 2023). This result was supported by the findings of Mohamed et al. (2025) who investigated the economic efficiency of feeding broilers with sunflower meal at 0% and 5% levels with and without lysine. In this trial, control diet (T1) contained 0% SM and the recommended amount of lysine, while the dietary treatments received 5% SM with different levels of lysine, where treatment 2 (T2) received same lysine content as the control while T3, T4, T5, and T6 offered 5%, 10%, 15% and 20% more lysine respectively. Their findings revealed that broilers offered 20% more lysine (T6) had a significantly higher net return, net profit and performance index compared to other treatments. Therefore, feeding SM supplemented with lysine offered economic benefits, enhanced feed efficiency and even improved performance that makes it a viable strategic alternative for broiler production.

Therefore, considering the higher price of soybean meal and its environmental problem due to long distance transportation, feeding locally available comparably cheap sources like SM as an alternative protein source is economically feasible and environmentally friendly.

3.4. Chemical composition of extracted sunflower meal

Sunflower meal is a by-product of sunflower seed oil extraction and is economically important as it can be applied as an alternative protein source in broiler feeds and is rich in sulphur amino acids but contain high fibre and is deficient in lysine (Chobanova, 2019). Sunflower seed (Helianthus annuus) consists of 25 to 40% shell, 65% total digestible nutrients (TDN), and the crude protein (CP) content varies, that is, if good quality high graded 35 - 44% CP, partially dehulled 34 – 38% CP, completely dehulled 40% up to 50% CP, and not dehulled 25 – 29% CP and considerably high fibre (Amrita, 2012; Pilorgé, 2020). The average nutrient content of sunflower meal (peeled), sunflower meal (partially shelled), and sunflower cake (partially shelled) are CP: 38.6, 33.3, 34.7; CF; 11.9, 19.7, 18.3; crude fat: 1.7, 2.2, 5.5; crude ash: 7.0, 6.2, 5.9; and sugar: 9.2, 6.0, 7.5 %, respectively (Zoltan Peter, 2018). The fibre components limit its use in poultry diets (Araújo et al., 2011) due to low NSP degradation in SM by chickens. The reason could be lack of endogenous NSPase, short retention time (7 to 15 hours) in the main fermentation cite of chickens, the ceca and only liquid or fibre sources with small particle size (less than 0.2mm) can pass and enter the ceca (Lannuzel et al., 2022). Tejeda and Kim (2021) also mention in their review, that ceca are the main organ where bacterial fermentation take place and a cite bacterial population and diversity is determined. However, the mentioned limitations of the chicken's digestive system hinder efficient fibre degradation in chickens that limits the use of SM in broilers. The oil residues (1 to 2%) in SM are rich in polyunsaturated linoleic acid (omega-6) and monounsaturated oleic acid (omega-9) (Papanikou, 2020).

According to different analytical reports, SM is categorized into three classes as un-dehulled, dehulled and double de-hulled or using the technological advancement to further dehull the already dehulled SM to produce low fibre-high protein SM (Lević et al., 2005; Papanikou, 2020a) . The undehulled SM contain 28 % to 30.5% CP and 23.5% to 28% fibre (Amrita, 2012; Attia et al., 2003; Ibrahim and El Zubeir, 1991; Pedrosa et al., 2000; Pilorgé, 2020) dehulled SM contain 33 – 40% CP and 15% to 21% fibre (Papanikou, 2020; Pedrosa et al., 2000; Villamide and San Juan, 1998), and the low fibre-high protein SM contain up to 44% CP and 10% to 15% fibre (Araújo et al., 2011; Lević et al., 2005; Papanikou, 2020; Tewelde et al., 2026a). Depending on the processing technique sunflower meal may contain 35% crude protein and 25% fibre (Ditta and King, 2017). However, following oil extraction, either all or part of the hulls are added back to the meal. The amount added back greatly influences the fibre content of SM, because hulls are rich in fibre (801 g/kg) relative to the cotyledon. The meal oil content also affects the concentration of fibre and proteins in the meal (Lannuzel et al., 2022).The partially dehulled type is the most widely available (Papanikou, 2020).

The defatted SM contains more sugar than the seed and the components of the dehulled defatted meal are 0.6% glucose, 2.3% sucrose, 3.2% raffinose and 0.8% trehalose. The hulls mainly contain cellulose, pentosan, lignin, and reducing sugars (Jithender et al., 2019). The chemical composition of SM considerably varied due to growing conditions, seed cultivar, different processing methods, the extent of dehulling of decortication, the efficiency of oil extraction and the age of the birds (Adesina, 2019; Levic et al., 2005) as shown in Table 1. As a result, Adesina (2019) reported that dehulling, solvent extraction, roasting, mechanical extraction and boiling showed a significant difference in the proximate components of sunflower seed meal as shown in Figure 1. Sunflower meal contains 11.2% fat, 5.32% ash, 20.94% nitrogen free extract (NFE), and 10.20 MJ/kg metabolizable energy (ME) (Babiker, 2012). All in all, the variety, oil extraction method, dehulling rate, and age of birds brings the inconsistency in the results of sunflower meal experiments in broilers.

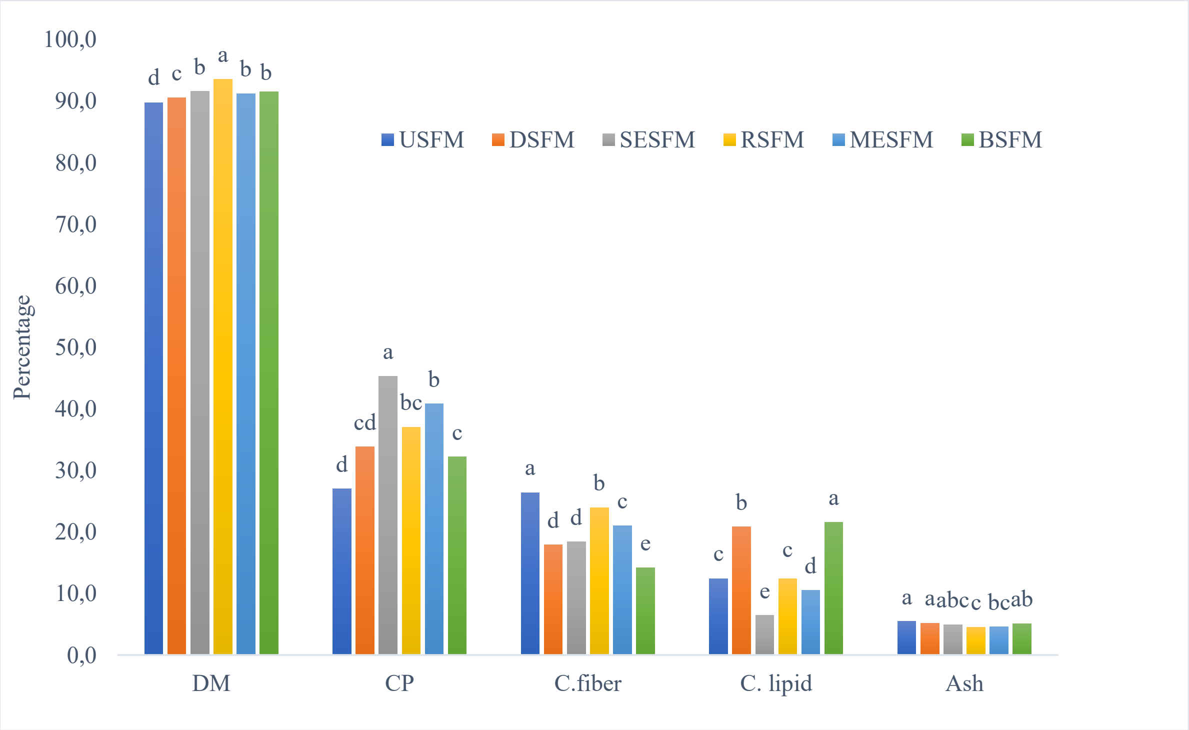

Figure 1. Processing effect on proximate composition of raw and differently processed sunflower seed meals (Adesina, 2019)

USM- un dehulled sunflower meal, DSM- dehulled sunflower meal, SESM-solvent extracted sunflower meal, RSM-roasted sunflower meal, MESM- mechanically extracted sunflower meal, BSM- boiled sunflower meal, DM- dry matter, CP- crude protein, C. fibre- crude fibre, C. lipid- crude lipid, $^{a, b, c, d}$ in the graph means parameters with different letters differ significantly

3.4.1. Protein, amino acid and mineral contents

The extracted sunflower meal is an important source of protein in animal nutrition. Sunflower meal protein concentration is similar to or higher than that of wheat and corn. Lysine is the most limiting amino acid in SM (Araújo et al., 2011; Mezőlaki et al., 2023, 2022), as a dietary protein for broilers, followed by isoleucine and cysteine (Levic et al., 2005; Mezőlaki et al., 2023). However, SM contains higher methionine, cysteine, and arginine than soybean meal. The amino acid (AA) composition of SM is also more balanced compare with soybean meal, wheat, and corn. Supplementation of L-lysine can considerably improve the amino acid content of sunflower meal protein (Araújo et al., 2011). The essential amino acids in SM are also close to the chicken's requirement (Mezőlaki et al., 2022). Supplementation of L-lysine can considerably improve the biological value of amino acid content in sunflower protein (Araújo et al., 2011; Levic et al., 2005).

Processing and method of extraction makes a huge difference in the protein and amino acid contents of sunflower meals and cakes. Oseyko et al. (2020) reported that, grinding sunflower press cake resulted in a substantial increase in protein content. Their results revealed that sunflower press cake flour had 22% and 31.1% higher in CP and essential amino acids respectively compared to the press cake from which the flour was isolated. The increase in CP is logically duet to the increase in amino acids in the sunflower flour obtained from sunflower kernel, an implication even physical processing of sunflower meal improves its nutritive content.

Table 1. Chemical composition of sunflower meals (%)

DM	CP	EE	CF	Ca	P	ADF	NDF	GE (Kcal/kg)	References
88.34	31.65	0.54	29.09	0.34	1.34	24.2	41.86	4324	(Aparecida Da Silva et al., 2012)
91.37	25	2.12	22.37	0.14	0.94	21.34	45.19	NA	(de Araújo et al., 2015)
92.7	27.5	3.08	25.9	0.22	0.67	32.90	43.57	NA	(de Carvalho Carellos et al., 2005)
91.6	35.1	1.46	21.9	0.4	1.04	25.4	33.2	4292	(Pereira and Adeola, 2016)
88	30	2.5	21.2	0.21	NA	NA	NA	4104	(Moghaddam et al., 2012)
89.26	32.49	1.93	25.05	0.32	0.88	28.97	43.51	4572	(Moghaddam et al., 2012)
91.5	34	1.06	26	6.71[†]	NA	31.2	40.92	NA	(Economides, 1998)
92.23	38.49	1.084	16.57	7.62[†]	1.389	20.25	28.38	4287.7	[a](Mezőlaki et al., 2022)
90.20	32.30	18.78	11.54	6.29[†]	NA	NA	NA	5017	[b] (Senkoylu and Dale, 2006)
91.48	33.52	3.11	27.23	0.32	0.94	28.9	44.87	NA	[C](Liu et al., 2015)

[†]= crude Ash, [a]= average of 20 representative extracted SM samples collected from the domestic market, [b]=high oil sunflower meal, c=the mean of 10 representative SM Samples, DM-dry matter, CP-crude protein, EE- ether extract, CF-crude fibre, Ca-calcium, ADF- acid detergent fibre, NDF-neutral detergent fibre, GE- gross energy, P- phosphorus

Table 2. The dispensable and indispensable amino acid contents of sunflower meal (% DM)

Essential amino acids												**Non-essential amino acids**						
ARG	**HIS**	**ILE**	**LEU**	**LYS**	**MET**	**PHE**	**THR**	**TRY**	**VAL**	**CYS**	**TYR**	**ALA**	**ASP**	**GLU**	**GLY**	**PRO**	**SER**	**References**
2.4	0.8	1.3	2.0	1.2	0.7	1.4	1.2	0.4	1.7	0.5	0.7	1.4	2.8	5.8	1.8	1.3	1.2	(Pereira and Adeola, 2016)
2.4	0.9	1.2	2.2	1.2	0.7	1.5	1.2	0.4	1.4	0.5	0.8	1.5	3.0	6.3	1.9	1.4	1.4	(Li et al., 2018)
3.7	1.2	1.5	2.6	1.6	0.8	1.9	1.5	NA	1.9	0.7	1.0	1.9	3.8	8.6	2.3	2.6	2.1	(Waititu et al., 2018a)
2.6	0.8	1.3	2.0	1.1	0.7	1.5	1.2	0.4	1.6	0.5	NA	1.4	2.9	6.1	1.9	1.4	1.3	(Wiltafsky et al., 2016)[⁑]
2.5	0.8	1.4	2.2	1.2	0.6	1.5	1.2	0.4	1.5	0.6	0.8	2.2	3.0	7.0	2.0	1.5	1.6	(Alagawany et al., 2015a)
2.6	0.9	1.3	2.1	1.5	0.8	1.4	1.3	0.4	1.8	0.6	0.8	1.6	3.0	6.8	2.0	1.2	1.4	(Liu et al., 2015) [†]
4.1	NA	1.9	3.3	1.6	0.7	2.4	2.9	NA	2.0	0.7	1.2	2.3	4.7	10.0	2.7	NA	2.6	(Ciurescu et al., 2019) [Θ]
2.2	0.7	1.2	1.8	0.9	0.6	1.3	1.1	NA	1.5	0.4	NA	1.2	2.6	5.5	1.6	NA	1.1	(Azam et al., 2019)
2.3	0.6	1.1	1.8	0.9	0.6	1.2	1.0	NA	1.4	0.3	NA	1.3	2.3	5.1	1.4	NA	1.2	
2.5	0.8	1.4	2.1	1.2	0.7	1.5	1.2	0.4	1.7	0.6	0.8	1.4	2.8	6.2	1.9	1.3	1.2	(Ibagon et al., 2021)[♦]
2.1	0.7	1.1	1.6	0.9	0.6	1.2	0.9	0.3	1.3	0.4	0.5	1.1	2.3	5.0	1.5	1.0	0.9	(Ibagon et al., 2021)[ω]
5.9	0.9	1.8	3.3	1.5	1.3	2.5	1.5	NA	2.2	0.1	0.8	2.0	0.8	1.5	2.4	2.2	1.9	(Oseyko et al., 2020)[#]
3.2	0.9	1.5	2.2	1.3	0.9	1.7	1.4	NA	1.8	0.6	0.8	1.6	3.4	7.3	2.1	1.5	1.6	(Such et al., 2024a)
3.3	1.0	1.6	2.5	1.4	0.9	1.8	1.5	NA	2.0	0.6	1.0	1.7	3.6	8.0	2.3	1.8	1.7	(Mezőlaki et al., 2022) *

[⁑]739 SM samples, [†]the mean of 10 representative SM samples, [Θ]low fiber sunflower meal, [♦] average of six sources of sunflower meal collected from Hungary, Ukraine, Italy and United states, [ω] sunflower expeller, [#]Sunflower press cake, *average of 20 representative extracted SM samples collected from the domestic market, NA–not available, ARG—arginine, HIS—histidine, ILE—isoleucine, LEU—leucine, LYS—lysine, MET—methionine, PHE—Phenylalanine, THR—threonine, VAL—valine, CYS—cystine, TYR—tyrosine, ALA—alanine, ASP—aspartic acid, GLU—glutamic acid, GLY—glycine, PRO—proline, TRY—tryptophan, SER—serine

Sunflower meal contains considerable amount of minerals such as Ca and P (Table 1) and other minerals as shown in Table 3. Sunflower seed cake contains 0.88% phytic phosphate (Wiltafsky et al., 2016), 0.06% silica (Gowda et al., 2004) and many other minerals. The addition of enzyme complex improves phosphorus and calcium retention in diets containing 20% SM (Tavernari et al., 2008).

Table 3. The mineral contents of sunflower meal

Minerals	(Gowda et al., 2004)	(Babiker, 2012)	(Falaye et al., 2016)	(Vasudha and Sarla, 2021)
Sodium	-	-	0.25%	-
Potassium	-	0.13%	2.01%	-
Calcium	0.34%	0.47%	0.17%	650mg/kg
Magnesium	0.28%	0.43%	0.17%	-
Phosphorus	0.83%	0.77%	1.57%	711.3 mg/kg
Iron	422 ppm	0.31 mg/kg	0.29%	6.44 mg/kg
Copper	24 ppm	34.08 mg/kg	0.01%	-
Zinc	64 ppm	93.83 mg/kg	0.11%	-
Manganese	-	32.17 mg/kg	0.15%	-
Molybdenum	-	1.22 mg/kg	-	-
Boron	-	69.54 mg/kg	-	-

3.4.2. Metabolizable energy content

One of the most important parameters to be considered while formulating poultry ration is energy content. Energy is very important for metabolism, physiological functions, maintenance, growth, tissue metabolism and heat production. In order to provide a reliable database with energy contents, a large data set containing apparent metabolizable energy (AME) or true metabolizable energy (TME) value of all feed staffs is necessary. However, there is no uniform and accurate system of assessing the AME value of feed ingredients yet (Chobanova and Penkov, 2022) and the same is true for sunflower meal. Sunflower meal can be used as a source of energy in broiler diets. Sunflower meal contains high non-phytate P, low total NSP, and high AMEn contents which make it an attractive alternative protein source for broilers (Waititu et al., 2018a).

Its ileal digestible energy (IDE) was found 8.17MJ/kg; ME ranges from 6.7 MJ/kg to 10.2 MJ/kg, (Babiker, 2012; Ciurescu et al., 2019; Pereira and Adeola, 2016; Saleh et al., 2021). Its AMEn value ranges from 6.09 to 6.51 (MJ/kg) (Attia et al., 2003; Nouri et al., 2008). The AME, and AMEn content varies with fibre content and processing methods of SM. As a result, the ME of SM expeller, SM solvent, and SM partially dehulled solvent is 9.67 (MJ/kg), 7.36 (MJ/kg), and 9.45 (MJ/kg), respectively (Batal and Dale. N., 2016). Similarly, its AME ranges from 11.20 to 11.25 (MJ/kg) (Azam et al., 2019), other findings reported that 8.05 (MJ/kg) (Waititu et al., 2018a). The AMEn content ranges from 6.50 to 10.32 (MJ/kg) (Azam et al., 2019; Levic et al., 2005; Waititu et al., 2018a) whereby its total AMEn was found 9.47 (MJ/kg) (Saleh et al., 2021), while the low fibre SM contains 7.09 (MJ/kg) (Ciurescu et al., 2019). Moreover, its IDE, AMEn was found 8.17 and 6.75 (MJ/kg), respectively (Pereira and Adeola, 2016).

Sunflower meal contains 6.75 (MJ/kg) TMEn in intact birds, and 6.53(MJ/kg) TMEn and 5.84 (MJ/kg) AMEn in the caecectomized birds. The removal of the ceca had no significant effect on ME of SM (Nouri et al., 2008). This may be because of the amount of insoluble polysaccharide contents of SM in ceca of intact cockerel (Kocher et al., 2000). Sunflower meal inclusion at 20% level increased AMEn of the broiler diets (Tavernari et al., 2008). High AME and AMEn of broilers fed diets containing SM with and without enzyme showed non-significant differences (Kocher et al., 2000).

3.4.3. Anti-nutritional compounds

Anti-nutritional factors, most common, in plant protein sources include toxic amino acids, saponins, cyanogenic glycosides, tannins, phytic acid, gossypol, oxalates, goitrogens, lectins, protease inhibitors, chlorogenic acid, and amylase inhibitors (Akande and Fabiyi, 2010). Hulls, flakes, expelled cakes and extracted meals are some of the sunflower oil processing by-products (Kreps et al., 2014). The hulls in SM are the components that contribute to high fibre content. Sunflower is almost free of toxic compounds that may impede their use in animal nutrition. However, the presence of high husk (non-starch polysaccharides), lysine and threonine deficiency (Chobanova, 2019) as well as its polyphenolic compounds such as chlorogenic acid content in the hulls may limit its application in poultry feeding. Generally, the dietary fibre fractions of SM as fed basis were found as follow: 152.7 g/kg CF, 270.2 g/kg NSP, 7.8 g/kg water soluble NSP, 262.4 g/kg water-insoluble NSP and 85.7 g/kg lignin. So, the total dietary fibre contained in SM is about 35% (Jankowski et al., 2011). Other findings reported that, 4.3% soluble NSP, 24.8 % insoluble NSP, and 14.4% cellulose as a fed basis (Amerah et al., 2015).

Sunflower seeds are rich in phenolic compounds principally chlorogenic acid, a tannin-like compound that inhibits the activity of digestive enzymes including trypsin, chymotrypsin, amylase, and lipase (Akande and Fabiyi, 2010). The total phenol content in sunflower seeds is in the range of 10 to 42 g/kg (Kreps et al., 2014). Phenols are 1 to 3% of the seed and are present in trace amount in cold-pressed sunflower seed oil (De Leonardis et al., 2003). Sunflower meal obtained after the extraction of lipids from cakes had similar content of phenolic antioxidants as sunflower seeds. In the production of sunflower oil, mainly in alkaline conditions, chlorogenic acid reacts with certain protein fractions, this gives the meal a characteristic, dark green colour (Kreps et al., 2014). Because chlorogenic acid is uncondensed and non-hydrolysable, its content of 1% or more of the total content of 3 to 3.5% phenolic compounds in sunflower meal is not reported in tannin assays. As it is the precursor of ortho-quinones that occur during plant enzyme polyphenol oxidase reacts with polymerized lysine during processing or in the gut. The toxic effects of chlorogenic acid can be counteracted by dietary supplementation with methyl donors like choline and methionine (Akande and Fabiyi, 2010).

Heating sunflower seed at 100 ºC, or at 135 ºC for 5 hours, destroyed about 43% of chlorogenic acid, however, excessive heating of SM may denature proteins and result in decreased amino acid levels, especially lysine and methionine (Casartelli et al., 2006). Heating high protein SM at 136 ºC for 20 minutes had no effect on nitrogen and AA digestibility, and pre-caecal flows except for darkening in colour (Elling-Staats et al., 2022). Chlorogenic acid can be readily removed from sunflower seeds using aqueous extraction methods that the aqueous process removes 88% of the chlorogenic acid initially present in the kernels and the diffusion of the defatted paste with hot water at short intervals is more efficient that removes 98.6% of this compound, finally, protein digestibility is enhanced after applying the diffusion technique (Domínguez et al., 1993).

Sunflower meal that contains polyphenols of 4270 mg/100g was processed with absolute ethanol, sodium sulphite 1%, deionized water, sodium bicarbonate 0.5%, sodium chloride 5%, calcium hydroxide 1%, methanol, acetone aqueous, and hydrochloric acid 0.5M. This processing method reduced polyphenol significantly. Moreover, washing of SM sample, that contains 1200 mg/100g polyphenols, with water of various pH reduced the polyphenol content of SM by more than 99% as shown in Table 4. Eventually, the proximate composition of SM resulted in reduction of phytates from 1.2% to 0.2% and the polyphenols from 4.27% to 0.3% (Gandhi et al., 2008). This indicates the significance of different treatments in reducing SM polyphenol content.

Table 4. Processing and washing effects of sunflower meal on polyphenol content reduction

Treatment	Polyphenol after processing (mg/100 g)	reduction in polyphenol in (%)	water pH for washing	polyphenol after washing (mg/100 g)	reduction in polyphenol content (%)
Absolute Ethanol	530	87.59	2	0.97	99.92
Sodium Sulphite 1%	220	94.85	2.5	0.94	99.92
Deionized Water	1740	59.24	3	0.8	99.93
Sodium Bicarbonate 0.5%	2594	39.25	3.5	0.66	99.95
Sodium Chloride 5%	1995	53.27	4	0.43	99.96
Calcium Hydroxide 1%	2905	31.97	4.5	0.23	99.98
Methanol	445	89.57	5	0.09	99.99
Acetone Aqueous	40	99.06	5.5	0.09	99.99
Hydrochloric Acid 0.5M	50	98.83	6	0.66	99.95

Source: (Gandhi et al., 2008)

A similar result was found that the application of multi-step washing of sunflower seed with greater than 60% aqueous or ethanol solution removes 95% of the phenolic compound and reduces the protein content of sunflower by 3%. The researchers reported that less than one percent protein loss can be achieved by using an 80% ethanol solution accompanied by increasing steps of washing (Jia et al., 2021).

3.5. Effect of feeding extracted sunflower meal on production traits and carcass composition

Replacing soybean meal with high-protein SM at 5% to 15%, 10% to 25%, and 15% to 26.5% in the starter (10 d), grower (24 d), and finisher (49 d) phases, respectively, did not show a significant difference in growth performance and FCR in all age groups. As a result, it was found this high-protein SM can provide up to 53% of the dietary CP required during the growers and finisher period

(Gerzilov and Petrov, 2022). The safe inclusion of high-protein SM in order to achieve a higher performance was 10%, 20%, and 23% in the starter, grower, and finisher periods, respectively (Yaqoob et al., 2022). However, the safe inclusion rate varies with the type of SM used, for instance, the safe inclusion rate in the case of sunflower cake is 10% during the finisher phase from 21 to 42 days of age (Berwanger et al., 2017b). Sunflower meal (0%, 10%, 20%) with two levels of NSPase supplementation (0 or 100 g/ton feed) replaced soybean for Ross-308 broiler chicks. Diets were formulated for starter (1 to 10 days), grower (11 to 21 days), and finisher (22 to 35 days). Sunflower meal up to 20% and NSPase supplementation had no effect on FI, BWG, and FCR on days 21 and 35 (Munawar et al., 2025).

Increasing levels of SM from 0, 7, 14 up to 21% increased feed intake quadratically during the grower (22 to 42day) and finisher (43 to 49 days) period as well as the overall period 1 to 49 days. Body weight gain also highly responded quadratically at starter, grower, and finisher and overall period and linearly for all except the finishers responded non significantly with increasing levels of SM (Moghaddam et al., 2012).

The replacement of soybean meal (SBM) by 5, 10 and 15% SM levels with and without enzyme (Table 6) resulted in 6.37, 6.05 and 6.4% improvement in BWG respectively when compared to control at 5weeks age. The highest weight gain was recorded at 5% supplemented with enzyme. Birds fed the diet without enzyme at 15% also showed highest weight gain and increased FI. Feed intake linearly increases with increments in sunflower supplementation from 5 to 15% level without enzymes. However, the gain in weight, feed intake, weight record and FCR in all levels were statistically non-significant (Desai et al., 2018).

Sunflower meal promotes an all-phase (1 to 42 day) feed: gain ratio and reduces feed intake at the starter phase and total experimental period. There was no significant effect of SM supplementation (0 and 20%) on the production traits such as carcass yield, abdominal fat, thigh and drumstick, breast, and breast fillet yields (Tavernari et al., 2008). Sunflower meal (50 g/kg) supplementation to broilers diet 1 to 21-day had a higher and more significant effect on feed intake compared with the control and SM 60 g/kg. Generally, SM supplementation had a positive effect on feed to gain ratio of growers and finishers (Amerah et al., 2015)

Yaqoob et al. (2022) investigated the effect of replacing SBM by SM with added multienzyme at 3, 6 and 9% levels on broilers growth performance and reported that there was no significant difference at all levels in feed intake, weight gain and FCR from 1 to 21 and 21 to 35-day age. Similarly, there was no significant difference in average BWG and FCR of replacing SBM by SM. But replacing SBM by SM at 50 and 75% levels significantly increased feed intake (Molale, 2020). Feed intake and BWG significantly and linearly decreased with increasing levels of SM. A linear decrease in FI, FCR and BWG with an increase in the levels of SM (4, 8, 12 and 16% levels) was reported (de Oliveira et al., 2016).

Incorporating10% and 15% high protein SM reduced live BW compared to the control. The reduction was significant at day 10 (10.4 and 11.8%), day 28 (2.5 and 4.1%) and day 42 (1.6 and 4.1%) for the 10 and 15% SM, respectively. However, non-significant were found for the 10% level at days 28 and 42. The retardation was higher at the starter phase with 15% SM level. Poor FCR was recorded at day 28, broilers weigh 3.9 and 5.6% less than the control and exhibit higher feed consumption per unit weight gain at 10 and 15% inclusion levels for the entire 0 to 42-day experimental period. Sunflower meal increased FCR by 2.7% and 4.2% of the experimental group with 10 and 15% levels respectively (Chobanova, 2019).

Sunflower meal at 7, 14 and 21% levels increased FI quadratically with increasing levels of SM for growers, and finishers. The overall 1 to 49 days improvement in FI, BWG and FCR responded quadratically to SM increments except for FCR of the starters had a non-significant effect (Moghaddam et al., 2012). Increased replacing SBM with SM from 25%, 50%, 75% up to 100% in the corn-soybean meal-based diet linearly decreased feed intake, body weight, and body weight gain at the starter (0 to 21d age), from days 21 to 35 age only FI and BWG showed a linear decrease with increasing levels of SM. The gain-to-feed ratio had no significant difference both at starter and finisher levels. But in the overall phases all FI, BW, and BWG show a linear decrease with increasing SM, the BWG also shows a quadratic decrease in gain (Waititu et al., 2018b).

The highest relative organ weight of breast, thigh and abdominal fat was recorded at 14% SM. And at 21% the highest weight of the GIT and gizzard were recorded. The thigh and liver responded quadratically, and the GIT and gizzard recorded a significant linear increase with an increase in SM levels (Moghaddam et al., 2012). The inclusion of SM at 0 and 15% had no effect on FI but WG had decreased significantly at SM 15% (1.646 g) compared to the control (1.824 g) (Araújo et al., 2011). Dehulled SM with a CP content of 38% was used at 7.5, 10, 13, 13.5% diets with a negative control, positive control of (extra 80 Kcal AME) and each supplemented with different enzymes and enzyme combination (Table 6) was examined at four feeding phases. The results showed that at starter and grower (1 to 20-d age), all the treatments applied did not show a significant difference in BWG, FI, and FCR due to diet and enzyme, except diets with XAP showed highest BWG. At the finisher phase (21 to 28-d), there was diet type, enzyme, diet and enzyme interaction on BWG. The enzyme XAP and X enzymes showed a higher significance difference on BWG than all other enzymes. At 35-d age SM 13.5% showed a higher and significant difference on BWG and FI compared to SM3%. Xylanase resulted in significantly higher BWG, FI and FCR. Therefore, X and XAP enzymes can improve BWG of broilers grown to 35 d (Mbukwane et al., 2022).

The use of sunflower meals at 15% level in broiler diet does not influence carcass or cut yields such as carcass, breast, legs, wings and back yields (Araújo et al., 2011). Generally, carcass percentage was numerically higher with birds fed different levels of SM (4, 8, 12 and 16% in the diet) compared to the control. There was no significant improvement in breast, thigh, leg, and abdominal fat but wings showed a linear and significant improvement to increased levels of SM. A multienzyme and SM interaction was reported for thigh and leg carcass % and SM without enzyme at 8 and 12% levels showed higher thigh and leg yield than the control and enzyme-supplemented SM (de Oliveira et al., 2016). The addition of SM and enzyme (Table 6) to the broiler diet had no effect on the carcass characteristics such as carcass recovery, abdominal fat pad, and breast meat yield. Sunflower meal at 100 g/kg rate increases the empty gizzard weight compared to the control (Amerah et al., 2015).

Sunflower meal supplementation at 15, 20 and 25% levels showed a non-significant effect on dressing percentage, breast meat yield, relative liver weight, intestinal length and relative heart weight. There was a significant quadratic increase in gizzard and intestinal weight, and a significant linear increase in thigh weight from SM 15 to 25% (Bilal et al., 2017). Sunflower meal inclusion at 200 and 300 g/kg had no effect on carcass weight, breast, leg or abdominal fat but enzyme addition (Table 6) improved breast weight at 300 g/kg SM (Mushtaq et al., 2009).

Soybean meal replaced by SM at 3, 6 and 9% had no significant effect on the carcass parts of broilers such as dressing percentage, breast, thigh, drumstick, wing and abdominal fat (Yaqoob et al., 2022). A similar result was reported by (Molale, 2020) who replace SBM with SM at 25, 50 and 75% levels and found that the interaction between SM and enzyme had no effect on meat quality parameters, carcass characteristics such as live BW, cold carcass weight, drumstick, thigh, wings, and breast (Table 6). The pH values of these meat ranged between 5.54 and 5.63, whilst water holding capacity was 2.13 g in SM75% to 2.63 g in SM50% levels and control diets.

The internal organs indexes of heart, liver, gizzard, thymus and spleen were also non-significant except the bursa showed a significantly higher index to SM (3, 6 and 9%) supplementation (Yaqoob et al., 2022).The replacement of SBM with 25, 50 and 75% of SM had a significant effect on hot carcass weight (Molale, 2020). The relative GIT and gizzard weights linearly increased, the liver quadratically increased and the thigh quadratically decreased to increased levels of SM (0,7,14,21%). No significant response on abdominal fat and breast weight was reported at 49-day age (Moghaddam et al., 2012).

The partial replacement of SBM by SM at 3, 6, and 9% levels did not show any significant difference in meat quality such as water holding capacity and cook loss percentages of the breast and thigh meat but pH at 24 h significantly increases with SM replacement (Yaqoob et al., 2022). Similarly replacing SBM with SM showed non-significant differences in meat quality parameters, carcass characteristics, and serum biochemical parameters (Molale, 2020).

3.6. Effects of exogenous enzymes on sunflower containing broiler diets

Enzymes enhance feed quality and consistency, improve nutrient digestibility, increase animal performance, and reduce the impact of anti-nutritional factors. Also support gut health and help limit the growth of harmful microorganisms. Enzymes added to the diet can be applied individually or in multi-enzyme blends throughout all growth stages of both ruminant and non-ruminant animals (Velázquez-De Lucio et al., 2021). Exogenous enzyme supplementation in the diets of monogastric animals help to hydrolyse the NSPs and improve energy use (Tavernari et al., 2008). Improving digestibility, by reducing the detrimental effects of anti-nutritional factors, improves the net benefit that broilers obtain from their food and can be best achieved by the use of enzymes in the broiler feeds. Protease enzyme reduces N load in the gastrointestinal tract (GIT) and improves feed utilization achieved by reducing the amount of substrate available to the pathogenic bacteria (Desai et al., 2018).

The addition of a multienzyme complex (pectinase, protease, phytase, glucanase, xylanase, cellulase and amylase at 4000, 700, 300, 200, 100, 40 and 30 U/g levels respectively) to SM (0, 4, 8, 12 and 16% in the diet) was examined. Supplementation resulted in notable improvements in weight gain and feed intake, with increases of 3.17% and 3.24% from day 1 to 21, and 2.65% and 3.5% from day 1 to 42, respectively compared to non-supplemented groups (de Oliveira et al., 2016).

The addition of exogenous enzyme (Table 6) to SM based diet had no significant difference on internal organ weight (liver, spleen, heart, gizzard, small intestine, large intestine), intestinal length (small and large intestine), carcass characteristics and meat quality parameter (drumstick, thigh, wing, breast, LBW, water holding capacity, hot and cold carcass weight, pH). These findings indicated that SM could replace SBM up to 75% (Molale, 2020).

Table 5. Type of commercial feed enzymes and target substrates

Enzyme	**Target substrate**	**Target feedstuff**
Phytases	Phytic acid	All plant-derived ingredients
β-Glucanases	β-Glucan	Barley, oats, and rye
Xylanases	Arabinoxylans	Wheat, rye, triticale, barley, fibrous plant materials
α-Galactosidases	Oligosaccharides	Soybean meal, grain legumes
Proteases	Proteins	All plant protein sources
Amylase	Starch	Cereal grains, grain legumes
Lipases	Lipids	Lipids in feed ingredients
Mannanases, cellulases, Hemicellulases, pectinases	Cell wall matrix (Fiber components)	Plant-derived ingredients, fibrous plant materials

Source: (Ravindran, 2013)

Enzyme addition (Table 6) to SM acts on NSP more effectively by cleaving polymeric chains, thereby improving nutritive value, reducing gut viscosity, facilitating nutrient absorption, and enhancing weight gain. Accordingly, in their study, inclusion of SM up to 13.5% from days 21 to 35 resulted in a 13% increase in BWG from days 22 to 28 and a 13.5% increase from days 29 to 35. Feed intake (FI) was also increased by 13.5% over days 21 to 35, attributable to enzyme supplementation (Mbukwane et al., 2022).

Table 6. Exogenous enzyme effects on production performance of chickens

Level of SM	Enzyme type	Results/effect	Ref
SM at 0, 5,10 and 15% replaced SBM with and without enzyme	Protease enzyme with protease activity 75,000 PROT/ g at 200 g/tone feed	-Protease + SBM result in higher LW (4.14% higher), FCR and higher profit margin than non-supplemented. - SM5% + protease recorded highest BWG and birds fed SM at 5, 10 and 15% SM replaced SBM with and without protease recorded higher BWG than the control at 5wks age	(Desai et al., 2018)
SM 0% and 20% with or without	cellulase, β-glucanase, xylanase, and phytase at 100 g/MT of feed	Resulted in non-significant SM x enzyme interaction on FI, WG, feed: gain ratio but improved WG during the first 21 day.	(Tavernari et al., 2008)
50 and 60 g/kg starters and 80 and 100 g/kg levels at growers and finishers period	100 g/tone diet enzyme (xylanase 1220 U/kg and glucanase 152 U/kg)	-Had no effect on BWG, feed intake and FCR of starters (1 to 21-d) and on feed intake and BWG of growers and finishers. -It is significantly lowering feed per gain at 22 to 42 and 1 to 42-day age.	(Amerah et al., 2015)
SM at 15%, 20% and 25%	* Zympex 008 NSPase at 0.5 kg/ton of diet	- Had no effect on FI and BWG from 1 to 42d age - Improved feed to gain ratio at 15 and 20% levels but not at 25% may be due to the higher dietary CF - NSP-ase addition had no effect on the carcass characteristics and internal organs' relative weights except for thigh meat yield % and relative gizzard, but intestinal weight increased significantly with increased levels of SM from 15 to 25%	(Bilal et al., 2017)
SM at 200 and 300 g/kg	Rovabio^{TM} enzyme at 0.5 g /kg (endo-1,4-xylanase and endo-1,3-glucanase)	SM x enzyme interaction at 300 g/kg SM improved BWG and gain-to-feed ratio during 1 to 21days and during 1 to 42-day gain to feed ratio improved by 5.53%, FI decreased by 4.67% but there was no enzyme effect on live performance or carcass response at any stage. -300 g/kg SM without enzyme depressed breast weight but compensated by enzyme supplement	(Mushtaq et al., 2009)
SM at 0, 4, 8, 12 and 16%	**0.2 g/kg of a multienzyme complex	-Enzyme significantly increased WG and FI but not FCR at 1 to 21 and 1 to 42-days -Increase in SM levels without enzyme showed significantly, linearly and quadratically decrease in WG and FI both at 1 to 21 and 1 to 42-d	(Levic et al., 2005)

SBM replaced SM 0, 25, 50, & 75%	Kemezyme complex at 500g/ton feed	-SM without enzyme significantly increased AFI with increased replacement levels and no significant difference in AWG and FCR (11 to 42-d) -Enzyme supplements had no significant effect on AFI, AWG and FCR, carcass characteristics and meat quality (11 to 42-d)	(Molale, 2020)
3, 7.5, 10, 13, 13.5% dehulled SM given to 1 to 9, 10 to 20, 21 to 28, 29 to 35 days age respectively	Phytase (P) -150, xylanase (X) + β-glucanase(B)-111, X-50, amylase + XP-100 and XBP-50 g/tone diet	Enzyme resulted in higher BWG and increased FI from 21 to 35-day age	(Mbukwane et al., 2022)
High protein SM replaced SBM at 75%	Enzymes A* and B*	-Had no significant difference among SM treatmentswith and without enzyme on final BW, BWG, FI and gain to feed ratio	(Waititu et al., 2018b)
35% SM Enzyme A + 35% SM Enzyme B +35% SM	†Enzyme A, 150 ppm †Enzyme B, 400 ppm	Enzyme had no effect on digesta viscosity, growth, feed intake, FCR, NSP concentration in the intestine and digestibility of NSP in the ileum but had improved the digestibility of soluble NSP in the jejunum	(Kocher et al., 2000)

*Zympex 008-is a multicomponent NSP enzyme which contains α-galactosidase, β-manna-nase, protease, amylase,β-glucanase, xylanase and cellulase, **multi-enzyme complex (pectinase -4000 u/g, protease -700 u/g, phytase -300 u/g, glucanase -200 u/g, xylanase -100 u/g, cellulase -40 u/g, and amylase -30 u/g), Kemzyme contains (carbohydrase, protease, cellulase, xylanase, glucanase, NSPases), Enzyme A* (xylanase-4,000 U/kg, α amylase-500 U/kg, protease-8,000 U/kg) and enzyme B* (cellulase-1,700, pectinase-1,100, mannanase-240, galactonase-30, xylanase-1,200, glucanase-360, α amylase-1,500 and protease-120 U/kg diet), †Enzyme A was cellulase-derived and contained cellulase (10,800 U/g), endo-1,3(4)-β-glucanase (21,400 U/g), and endo-1,4-β-xylanase (37,700 U/g) activities, with minor pectinase side activity (177 U/g, , and Enzyme B was *Aspergillus aculeatus*-derived contained endo-1,3(4)-β-glucanase (54.7 fungal xylanase units/g), hemicellulase (15,000 viscosity hemicellulase units/g), and pectinase (3,017 U/g,.

Enzyme supplementation, Hamecozyme (xylanase, β-glucanase, phytase) and Rovabio (xylanase, β-glucanase, cellulase, arabinofuranosidases) enzymes at 500 g/ton rate improved the productive performance of birds (0 to 6 weeks age) fed sunflower-corn-based diets. Enzyme supplementation to SM significantly increases growth rate and FCR (Khan et al., 2006). Enzyme supplement (Table 6) improves the nutritional value of high protein SM and enables the inclusion of SM up to 75% in place of soybean meal, in broiler diets, without affecting productivity and barn hygiene management (Waititu et al., 2018b). It was concluded that SM can be used as an alternative cheaper dietary protein replacing SBM up to 75% with or without enzyme starting from 10 to 42-day age of broilers (Molale, 2020; Waititu et al., 2018).

The addition of enzymes had no effect on digesta viscosity, growth, feed intake, FCR, NSP concentration in the intestine and digestibility of NSP in the ileum but had improved the digestibility of soluble NSP in the jejunum (Kocher et al., 2000). Birds fed SM10% with enzyme NIBGE (NSP-degrading multi enzyme) at 1.5 L/100 kg feed and GRINDAZYM™ (xylanase + β-glucanase) at 0.5 kg/ton) showed a higher weight gain and FCR when compared with birds fed SM10% without an enzyme. Enzyme supplementation had no effect on feed intake (Raza et al., 2009). Similarly, (Ciurescu et al., 2019) reported that the addition of 0.2 g/kg microbial phytase had no effect on BWG, FI and FCR. Therefore, different research outputs revealed that enzyme supplementation improves BWG; (Khan et al., 2006; Mushtaq et al., 2009; Tavernari et al., 2008), FCR (Khan et al., 2006) and feed intake (de Oliveira et al., 2016; Khan et al., 2006; Mushtaq et al., 2009). Also, Raza et al. (2009) concluded broiler chicks can grow faster and more efficiently on a diet containing fibre-degrading enzymes than on a diet without these enzymes.

From the above-gathered information, the use of exogenous enzymes is important as they hydrolyse NSP that can potentially be used by birds, improving the energy use, feed conversion ratio, feed intake, reducing the viscosity of gut contents, reducing the excreta moisture and improving the conditions of the litter. However, there are a lot of contrasting research outputs in the use of SM and enzyme supplementation. (Mbukwane et al., 2022) indicated that there is a knowledge gap and inconsistency on the optimum inclusion rate of SM and best enzyme combinations for optimum results in poultry nutrition.

In addition, the use of high dose phytase may provide considerable benefits, often exceeding those expected solely from improvements in phosphorus digestibility (Cowieson et al., 2011).

Phytic acid, also known as inositol hexaphosphate, is a poorly soluble compound found in high amounts in broiler diets because it is a natural component of plants. Approximately 70% of the total phosphorus in these diets exists as phytic acid, yet broilers that are not supplemented with enzymes are able to utilize only about 20–30% of it. As a result, additional phosphorus sources, such as dicalcium phosphate, must be included in the feed. However, supplementing with extra phosphorus raises feed costs and contributes to environmental problems through the excretion of excess phosphorus and the depletion of non-renewable phosphate reserves. In addition to enhancing phosphorus digestibility, phytase supplementation also increases the availability of nutrients that are otherwise bound to phytic acid, thereby improving animal performance (Fernandes et al., 2019). As a result, recent research approaches suggest that improving digestibility should not focus solely on NSP-degrading enzymes. 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.

The main effect of phytase favours the availability of the nutrients that would be complexed to the phytic acid molecule. Therefore, resulted in improvement of animal performance. The reason for the improvement is that Phytic acid has a strong binding affinity to nutrients such as soluble proteins at low pH, amino acids, minerals, and starch (Yu et al., 2012). In case of SM, it is even more effective, because research has shown that the high phytate content (3 – 4% of dry matter, or 80 – 85% of phosphorus) of SM decreases the digestibility of nutrients and utilization of phosphorus in poultry (Krum, 2023). In the case of soybean-based diets, phytase supers-dosing supplementation was used effectively in some research, where the focus of its effectiveness is not only the higher availability of phosphorus, but an additional physiological effect of phytase super-dosing (Fernandes et al., 2019).

In a study high protein SM was added in to broilers diet at proportions of 0.05%, 0.10%, and 0.15% of the total phosphorus, replacing corn-starch with or without an enzyme phytase (500 FTU/kg). Their result showed that the enzyme supplemented SM-based-diet had improved apparent ileal digestibility , BWG, feed efficiency, and bone traits (Kim et al., 2019). The addition of 0.2 g/kg microbial phytase had no effect on BWG, FI and FCR (Ciurescu et al., 2019). In a soybean-based diet, Poernama et al.

(2021) concluded that their findings confirmed the efficacy of phytase and its synergistic effect with NSPase, as birds showed further improvements in growth performance following NSPase supplementation in the presence of phytase.

In the study of Lee et al. (2017) corn-soybean based diet was fed, at day 42 of experiment, the beneficial effect of phytase super dosing (1,500 FTU/kg) on feed intake and body weight gain was evident in the diets with low P dose. Although the super dosing reduced feed conversion rate (FCR) at all P levels ($P < 0.05$), this effect was more pronounced on the low P diet. Similarly, in the study of Paul et al. (2025) the phytase super dosing (2,000 FTU/kg feed) alone using maize-soybean meal-based diets delivered better feed efficiency in broiler chickens compared to the control or antimicrobial growth promoters contained diets.

The study by de Léo et al. (2026) investigated the effects of phytase super-dosing in broiler diets based on corn, soybean meal, and meat and bone meal. The researchers evaluated growth performance, carcass yield, pectoral myopathies, bone mineral composition, and the metabolizable of energy and nutrients. Higher phytase supplementation levels (2,000 phytase units/kg feed) resulted in increased weight gain and improved feed conversion ratio. The study concluded that, in broiler diets containing mixed phosphorus sources, including animal-derived ingredients, high-dose phytase supplementation can be an effective strategy to enhance feed efficiency and nutrient utilization.

In the study of Wolfrum et al. (2025) investigated the effects of dietary phytase on amino acids digestibility in broiler chickens in case of 50 % soybean meal, 20 % rapeseed meal, 20 % sunflower meal, and 10 % rice bran. According to their conclusion the phytase supplementation at 1,500 FTU/kg or higher does not limit the pre-caecal AA digestibility with increasing oilseed meal-rice bran level. In a study that examined separately the soybean, the sunflower (300 g/kg) and rapeseed meal to determine phytase effects on prececal amino acid (AA) digestibility and phytate ($InsP_6$) breakdown, prececal InsP6 disappearance was significantly affected by interactions between oilseed meal, inclusion level, and phytase supplementation. Overall, prececal InsP6 disappearance was higher in soybean meal diets (52%) than in sunflower meal diets (38%) and intermediate in rapeseed meal diets (43%). The average increase in AA digestibility was 7 pp in rapeseed meal, whereas it was 3 pp in sunflower meal and 1 pp in soybean. Because AA digestibility in the absence of phytase was lower in rapeseed meal and SM than in SBM, phytase supplementation reduced the differences in AA digestibility levels among oilseed meals although they did not cease it (Krieg et al., 2020).

Based on the information presented above, super-dose phytase supplementation can increase the nutrient availability from SM by breaking the protein-phytate and starch-phytate complexes, there by increasing the amount of protein and carbohydrates available for absorption by the animal. As a result, animal performance may be improved.

3.7. Effects of feeding extracted sunflower meal on nutrient digestibility

Digestibility is the fraction of a nutrient ingested that is absorbed by the bird and is not excreted in the faeces. It can be calculated by measuring nutrient consumed and nutrients in faeces (Blok et al., 2017; Hoehler et al., 2006; Lemme et al., 2004). To meet the amino acid requirement, determination of AA digestibility is vital (Barua et al., 2021).

Amino acid digestibility is influenced by multiple factors related to both diet composition and animal characteristics. AA digestibility varies among protein sources, can be affected by fiber and antinutritional factors contents of the feed, birds age, physiological conditions, poultry types, methodological differences in assay procedures and analytical techniques as well an interaction among ingredients within the diet (Blok et al., 2017).

Accurate diet formulation for meat chickens requires information on digestibility than the gross amino acid contents of dietary ingredients (Kadim et al., 2002; Waititu et al., 2018a). Digestibility tests are separated into two main categories, as excreta and ileal digestibility analyses. Ileal digestibility is gaining increasing attention in the feeding of poultry (Blok et al., 2017; Rodehutscord et al., 2004). Because, to evaluate a feed, the measure of AA availability is more accurate in the terminal ileum compared to the measurements taken from the excreta (Ravindran et al., 1999; Such et al., 2024a).To measure excreta digestibility samples can be collected from intact or caecectomised birds and for measurement of ileal digestibility, digesta could be collected from the distal part of the ileum (Lemme et al., 2004).

The main disadvantage of excreta digestibility based on precision feeding assay are, the excreta contain amino acids from urine and faeces as the urine and faeces are excreted mixed, in addition it ignores the effect of hindgut microbes on protein digestion, utilization and the microbial protein mixed to the amino acid profile and concentrations in the faeces. In this case such error sources can be eliminated by caecectomising the birds (Hoehler et al., 2006) and determining ileal or pre-caecal digestibility (Ravindran et al., 1999), where digesta are pooled from the distal part of ileum that avoids urine AA and effects of the hindgut (caecum) microbial fermentation. Estimates are determined by allowing the bird's normal feed intake behaviour and are additive when used in feed formulations (Lemme et al., 2004). Standard ileal digestibility (SID) and apparent ileal digestibility (AID) of AAs measured by different researchers are shown in Table 7.

Table 7. Standard ileal digestibility and apparent ileal digestibility of amino acids in broiler chickens fed sunflower meal

	SID%						**AID%**
Amino acids	(Wiltafsky et al., 2016)	(Waititu et al., 2018a)*	(Lemme et al., 2004)	(Ullah et al., 2016)	(Azam et al., 2019)		(Waititu et al., 2018a)*
Arginine	2.39	90.7	93	90.9	90	82	89.2
Histidine	0.69	80	88	77	86	79	65.2
Isoleucine	1.15	84.1	89	83.9	82	77	80.7
Leucine	1.77	79.2	88	82.1	80	79	76.7
Lysine	0.98	79	87	78.3	76	75	75.1
Methionine	0.65	91	92	91.4	90	84	88.5
Phenylalanine	1.31	84.1	90	78.9	77	73	82.5
Threonine	0.95	79.9	82	73.1	71	68	74.8
Tryptophan	0.37	NA	87	NA	NA	NA	NA
Valine	1.37	81.1	87	78.7	77	70	77.4
Cysteine	0.42	70.1	80	69.4	67	60	66.4
Met + Cys	1.07	NA	87	NA	NA	NA	NA

NA= not available, *= high protein SM,

In a study, soybean meal was replaced by SM at levels of 3, 6 and 9%, plus an exogenous multienzyme supplement at a rate of 100 mg/kg. The enzyme source used was Axtra XAP 101(20000 U/g of 1,4-β-xylanase, 2000 U/g of α-amylase, and 40,000 U/g of protease). The addition of these enzymes to SM-based diets showed a significant increase in broilers' ileal protein digestibility at 3 and 6% levels. However, failed to affect ileal digestibility of DM, EE, and CF (Yaqoob et al., 2022). In other study high protein SM was added in to broilers diet at proportions of 0.05, 0.10, or 0.15% of the total phosphorus, replacing corn-starch with or without an enzyme phytase (500 U/kg). Enzyme supplemented SM-based diet had improved AID, BWG, feed efficiency, and bone traits. Bone trait improvement was mainly because of the high bioavailability of P in sunflower meal (Kim et al., 2019).

Ileal digestibility coefficients and the AME and AMEn contents of corn-soybean meal-based broiler diets at the age of 15 to 22-days showed a linear decrease with increasing SM levels from 100 g/kg to 200 g/kg (Pereira and Adeola, 2016). The replacement of 75% of soybean meal with high protein SM, with or without enzyme, did not affect the AID% of DM, N, GE, and the jejunal digesta viscosity as well as the excreta moisture content percentages at 7 and 14-d age. However, excreta moisture reduced and showed a significant difference between the corn-soybean meal-based control diet and high protein SM supplemented with enzymes xylanase (4000 U/kg), amylase (400 U/kg) and protease (8000 U/kg per kg diet) at 21-d age (Waititu et al., 2018b). The addition of NSPase (500 g/ton) to SM at 15, 20, and 25% levels had no effect on DM, CP and EE digestibility but improved feed to gain ratio at 15 and 20% SM levels and broilers were also able to break down the dietary CF up to 6 to7% without affecting FCR, however, increasing SM to 25% deteriorated FCR (Bilal et al., 2017).

3.8. Effects of feeding sunflower meal on gut morphology and digesta viscosity

The rapid growth of chickens is directly related to the morphological and functional integrity of the digestive system. Intestinal villus height (VH) and density are the indicators of the absorptive capacity of the intestinal mucosa (Gopinger et al., 2014). A shortening of the villus and a large crypt can lead to poor nutrient absorption, increased secretion in the gastrointestinal tract, diarrhoea, reduced disease resistance, and lower overall performance (Moghaddam et al., 2012). A higher villus increases the area of contact of the enterocytes with the digesta, resulting in a surface area increment for the absorption of nutrients (Gopinger et al., 2014). At the same time, the crypt can be regarded as the villus factory, and a larger crypt indicates fast turnover of tissues and an increased need for new tissue generation (Moghaddam et al., 2012).

The addition of a multienzyme complex (pectinase, protease, phytase, glucanase, xylanase, cellulase and amylase at 4000, 700, 300, 200, 100, 40 and 30 U/g levels respectively) to SM (0, 4, 8, 12 and 16% in the diet) significantly increases VH in the duodenum and jejunum, and significantly decreased the crypt depth (CD) in the duodenum, jejunum, and ileum (de Oliveira et al., 2016). This indicates that enzyme supplement can enhance the nutrient absorption, decreases secretion in the gastrointestinal tract, minimizes the occurrence of diarrhoea, increases disease resistance, and improve overall performance. In contrary an iso-nutritive corn soybean meal-based diet was supplemented by sunflower cake at 0, 5, 10, 15 and 20%. The result revealed a linear decrease in villus height, linear increase in crypt depth and linear decrease in villus to crypt ratio with an increase in sunflower cake levels fed to broilers at 21day of age. The addition of an enzyme complex composed of pectinase, protease, phytase, glucanase, xylanase, cellulase and amylase at 4000, 700, 300, 200, 100, 40 and 30 U/g respectively, applied at 0.02% of the total diet, failed to make a significant change compared to the diet without enzyme(Berwanger et al., 2017a).

Soybean meal was replaced by SM supplemented with multienzyme (100 mg/kg) at 0, 3, 6, and 9% levels. The source of enzyme was Axtr XAP (xylanase, amylase and protease at 20000, 2000 and 40000 U/g, respectively), manufactured by DuPont. The highest VH 1221.6 µm and 1187.6 µm for 3% SM and control (0% SM) respectively were the results of jejunal morphology and there was a non-significant difference among the control, 3%, and 6% SM supplementation. The lowest VH/CD ratio was found at 9% SM and the highest in the control. However, the control and 6% SM were statistically non-significant. The CD was higher at 3% SM and statistically non-significant for 0, 6, and 9% SM inclusion levels. Jejunum and duodenum length and their weight showed an increasingly significant difference when soybean is replaced by 3% SM (Yaqoob et al., 2022).

In the duodenum villus height, crypt depth and villus height to crypt depth ratio show significant linear and quadratic responses due to increasing levels of sunflower meal from 0 to 21%. For the jejunum, the response due to increasing levels of SM responds linearly and quadratically for villus height, quadratically for crypt depth and VH:CD but had no effect on villus width. In the ileum, there was neither linear nor quadratic response on VH, CD, VH:CD, and VW due to an increase in SM (Moghaddam et al., 2012). Increasing SM levels (7, 14 and 21%) showed both linear and quadratic responses in the duodenum where VH and VH:CD decreased with increasing levels of SM and crypt depth increased with an increase in SM levels. In the jejunum, VH decreased linearly and quadratically when SM increased from 7 to 21%. However, there was a positive quadratic response for crypt depth and VH:CD but had no effect on villus width. In the ileum, there was neither linear nor quadratic response on VH, CD, VH:CD, and VW due to an increase in SM levels (Moghaddam et al., 2012).

Regarding the digesta viscosity, replacement of soybean meal with SM at 0%, 10%, and 20% in the diet increased digesta viscosity significantly with increasing levels of SM (Munawar et al., 2025). Sunflower meal that contained 29.1% crude fibre (CF), was included at 0%, 5%, and 6% in the diet during the starter phase (1–21 days) and at 0%, 8%, 10% during the grower or finisher phases (22–42 days) with and without an enzyme (100 g/ton enzyme; xylanase, 1220 U/kg, and glucanase 152 U/kg). These diets were fed ad libitum and had no effect on jejunal digesta viscosity (Amerah et al., 2015). Sunflower meal with 18.5% CF was fed at a rate of 16% to pullets and 20% to laying hens, which significantly reduced jejunal viscosity compared to the control diet. Jejunal viscosity was also significantly higher in laying hens than pullets (Such et al., 2024b).

Similarly, digesta viscosity increased when 15% of SM was added to the control diet (Ditta and King, 2017). A corn-casein-based broiler diet was replaced with 35% SM with and without enzymes A or B. Enzyme A contained cellulase, glucanase, xylanase and pectinase and enzyme B contained glucanase, xylanase, hemicellulose, viscosity hemicellulose and pectinase. The enzymes were added directly at a rate of Enzyme A, 150 ppm and enzyme B, 400 ppm in the diet. Supplementing the enzymes failed to improve ileal or jejunal viscosity (Kocher et al., 2000). Enzyme supplementation to high protein SM and SM supplementation to broilers' diet resulted in no effect on the jejunal digesta viscosity, may be because of the low NSP content of high protein sunflower meal (Waititu et al., 2018b). Sunflower meal affects enzyme activities of amylase and protease significantly (0.05%) when SM replace SBM and the highest activities of amylase and protease were recorded for birds fed 75% SM diets compared with 0, 25 and 50% (Alagawany et al., 2017).

Feeding SM at 0%, 6%, and 8% for growers and at 0%, 10% and 16% for finishers with and without the enzymes (cellulase, β-glucanase, and xylanase at 0.01%) was studied by Horvatovic et al. (2015). As a result, the enzyme decreased digesta viscosity, and SM without enzyme increased ileal digesta viscosity. Enzyme addition to SM acts up on NSP more effectively by breaking the polymeric chain, thereby improving the nutritive value, reducing viscosity, facilitating nutrient absorption and improving weight gain. As a result, in their study, the inclusion of SM up to 13.5% resulted in higher BWG (13% and 13.5%), and FI (13.5%) due to enzyme supplementation (Mbukwane et al., 2022).

4. MATERIALS AND METHODS

4. 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.

4.1. Materials and methods applied in both experiments

4.1.1. 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. The campus ethics committee approved it in accordance with animal welfare legislation under the licence number MAB-1/2023. The experimental house is an environmentally computer controlled illuminated house. As a result, the environmental conditions of the different pens within the house such as heating, lighting, and ventilation were identical. Birds in each pen shared the same feed and water as a group. A balanced feed and automatic drinking water were supplied ad lib. Each pen was provided with clean wood shavings as a bedding material, and an adequate area for exercise (14 birds/m^2).

In experiment 1, a total of 624-day old male broiler chicks (Ross 308) with a body weight of 45.6 ± 0.4 g were purchased from a local hatchery (Gallus Ltd., Devecser, Hungary). Then 600 birds were randomly allocated to 25 experimental pens. There were five treatments with 120 chickens in each treatment. Each treatment had five replicates each containing 24 birds. 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). Birds received 24, 23, 20, and 18 lighting hours on day 0, days 1 to 7, days 8 to 11, and days 12 to 38, respectively. Each bird was vaccinated against infectious bronchitis (CEVAC BRON), Newcastle disease (VITAPEST), and infectious bursal disease (CEVAC TRANSMUNE) in the hatchery. The vaccine is produced by Ceva (Ceva Sante Animale, Libourne, France).

4.1.2. 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).

4.1.3. Digestibility and metabolizable energy calculations

The following equation were applied for the faecal nutrient digestibility and AA digestibility calculations:

$$\text{Nutr. digestibility } = \left(\frac{\text{Nutr.}_{\text{diet}} - (\text{Nutr.}_{\text{excreta}}\ (\text{TiO}_{2\ \text{diet}} / \text{TiO}_{2\ \text{excreta}}))}{\text{Nutr.}_{\text{diet}}} \right) \times 100$$

where

Nutr. digestibility = nutrient digestibility (%);

$\text{Nutr.}_{\text{diet}}$ = nutrient content of the diet (%);

$\text{Nutr.}_{\text{excreta}}$ = nutrient content of the excreta (%);

$TiO_{2\ \text{diet}}$ = TiO_2 content of the diet (%);

$TiO_{2\ \text{excreta}}$ = TiO_2 content of the excreta (%).

$$\text{AAD} = \left[\frac{\text{AA}_{\text{diet}} - \ (\text{AA}_{digesta} \times \ (\text{TiO}_{2\text{diet}} / \text{TiO}_{2\ \text{digesta}}))}{\text{AA}_{\text{diet}}} \right] \times 100$$

Where,

$AAD_{=}$ Amino acid digestibility of the diet (%);

AA_{diet} = Amino acid content of the diet (%);

$AA_{digesta}$ = amino acid content of the intestinal part digesta (%);

$TiO_{2\ diet}$ = TiO_2 content of the diet (%);

$TiO_{2\ digesta}$ = TiO_2 content of the intestinal part digesta (%).

$$\text{Nitrogen ret.} = \left[\frac{N_{diet} - (N_{excreta} \times (TiO_{2diet}/TiO_{2\ excreta}))}{N_{diet}}\right] \times 100$$

where

Nitrogen ret. = nitrogen retention (%);

N_{diet} = nitrogen content of the diet (%);

$N_{excreta}$ = nitrogen content of the excreta (%);

$TiO_{2\ diet}$ = TiO_2 content of the diet (%);

$TiO_{2\ excreta}$ = TiO_2 content of the excreta (%).

$$AME = GE_{\ diet} - (GE_{\ excreta} \times \left(\frac{TiO_{2\ diet}}{TiO_{2\ excretra}}\right)$$

where

AME = apparent metabolisable energy (KJ/g);

GE_{diet} = gross energy content of the diet (KJ/g);

$GE_{excreta}$ = gross energy content of the excreta (KJ/g);

$TiO_{2\ diet}$ = TiO_2 content of the diet (g/kg);

$TiO_{2\ excreta}$ = TiO_2 content of the excreta (g/kg).

$$N_{retention} = N_{diet} - \left(\frac{TiO_{2\ diet}}{TiO_{2\ excreta}}\right) \times N_{excreta}$$

where,

$N_{retention}$ = nitrogen retention (gN/g diet);

N_{diet} = nitrogen content of the diet (gN/g diet);

$N_{excreta}$ = nitrogen content of the excreta (gN/g excreta);

$TiO_{2\ diet}$ = TiO_2 content of the diet (g/kg);

$TiO_{2\ excreta}$ = TiO_2 content of the excreta (g/kg).

$$\text{AMEn (kJ/kg)} = \text{AME} - (\text{N}_{\text{retention}} \times 34.4)$$

where

AME_n = apparent metabolisable energy nitrogen corrected zero N retention;

AME = apparent metabolisable energy (KJ/g);

$\text{N}_{\text{retention}}$ = nitrogen retention (gN/gdiet);

34.4 = constant, the GE value of uric acid (KJ/g N).

4.1.4. 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). After thawing, samples were mixed 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.

4.2. Materials and methods of the first experiment

The aim of the first experiment is 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 parameters, nutrient digestibility and amino acid digestibility dynamics in different parts of the small intestine on day 27 and 41.

4.2.1. Experimental diets

The nutrient contents of high fibre sunflower meal (HFSM), and low fibre sunflower meal (LFSM) are shown in Table 8. High fibre SM contained less protein, amino acids, but more structural and insoluble fibre. On the other hand, LFSM contained more protein, amino acids, and less insoluble fibre. The soluble fibre fraction of LFSM was higher than that of HFSM.

Table 8. Measured nutrient content of sunflower meal (g/kg)

Nutrients	**HFSM**	**LFSM**
Dry matter	901.9	899.3
Crude protein	383.9	438.4
Crude fat	5.2	11.4
Crude fibre	166.8	103.5
Ash	67.3	76.8
Starch	24.5	26.5
Neutral detergent fibre	249.2	200.2
Acid detergent fibre	195.1	141.1
Insoluble dietary fibre	341.2	248.5
Soluble dietary fibre	79.7	87.2
Arginine	6.7	7.0
Histidine	36.4	41.2
Isoleucine	9.1	11.0
Leucine	15.1	17.5
Lysine	17.2	20.0
Methionine	79.2	93.8
Phenylalanine	17.1	20.8
Threonine	23.3	27.2
Tyrosine	17.3	19.9
Valine	20.8	21.5
Alanine	16.6	17.8
Aspartic acid	25.1	27.4
Cystine	10.0	10.9
Glutamic acid	18.3	20.4
Glycine	10.1	13.0
Proline	14.2	16.5
Serine	33.1	36.4

HFSM—High-fibre sunflower meal, LFSM— Low-fibre sunflower meal.

The composition of the experimental diets is shown in Table 9. 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 two forms of SM were incorporated mainly at the expense of soybean meal, and the lower AMEn content of SM was compensated with more sunflower oil (Table 9). The measured nutrient content of the diets shows that the values were close to the predicted ones (Table 10).

Table 9. Composition of the experimental diets (g/kg)

Ingredients	**Starter**	**Grower**					**Finisher**				
		C	**HFSM 20**	**HFSM 30**	**LFSM 20**	**LFSM 30**	**C**	**HFSM20**	**HFSM30**	**LFSM 20**	**LFSM 30**
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
Sodium Bicarbonate	1	1	1	1	1	1	1	1	1	1	1
Lysine (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 10. 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.

4.2.2. Samplings and measurements

4.2.2.1. 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. Body weight gain (BWG) was then calculated by deducting the initial average BW from the final average BW. FCR was calculated based on grams of feed consumed to produce a gram weight gain.

To measure the digestibility of the diets, 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. Each cage was equipped with automatic drinkers and feeders. After 2 days of adaptation (on days 26 and 27, and days 40 and 41) representative excreta samples were collected, avoiding contamination with feed and feathers.

The daily samples were stored in a refrigerator. On the second day 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.

4.2.2.2. Viscosity measurement

Stored samples were thawed overnight in room temperature, 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) with a CP40 cone and shear rate of 60 – 600 s^{-1}. Samples were centrifuged after every three-sample analysis. After centrifuging, 0.5 mL supernatant was taken using an adjustable volume pipette-man and poured into the cup. The sample-containing cup was then assembled with the adjustment ring and fitted with the cone spindle for viscosity analysis. 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. For reading the results with ± 1% accuracy range and ± 0.2% repeatability a special software was used (DV-Loader V2.1). The viscometer was connected to a temperature bath to maintain temperature at 25 °C for precise and reproducible viscosity measurement. Viscosity measurements were expressed in centipoise (cPs) units (1cPs – 1/100 dyne sec/cm2 = 1mPa.s) prior to statistical analysis.

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

From the excreta samples their 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.

4.2.3. 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 analysed using Shapiro–Wilk test 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 $p < 0.05$.

4.3. 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.

4.3.1. Experimental diets

The composition of the experimental diets is shown in Table 11. Four iso-nitrogenous and iso-caloric diets were formulated according to the breeder's recommendations (Aviagen, 2018). The four 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/kg), the C diet supplemented with higher dose of phytase (1500 FTU/kg) (CP), a sunflower meal-based diets (SM) and SM-based diet with higher dose of phytase (1500 FTU/kg) (SMP). 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 SM used in this experiment was slightly higher in crude fibre content (19% CF), and lower in crude protein content (35.5% CP) compared with the high fibre SM (HFSM) used in the first experiment. It contained 88% dry matter, and 3.5% crude fat.

Table 11. 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).

The analysed nutrient content of the experimental diets is shown in Table 12. The measured nutrient contents were close to the predicted values.

Table 12. 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	887.1	898.8	897.1
Crude protein	244.7	199.1	200.6	207	206.2	187.8	182	187.1	188.6
Crude fat	73.1	72.8	73	102.5	107.5	75.2	74.1	120	124.4
Crude fiber	41.7	44	39.8	92.2	93	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	9.9	10.1	9.8	9.1	8.8	8.9	9.2
Phosphorus	7.7	7	7.2	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	40.7	38.9
ARG	16.0	12.9	13	14.1	14.4	11.7	11.5	13.6	13.4
ILE	10.1	8.3	8.2	9.1	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	07.1	5.5	5.6	5.8	5.8	5.2	5.2	5.8	5.8
THR	09.9	8	8.5	9.3	9.6	8.6	8.3	8.8	8.7
VAL	12.2	9.6	9.4	9.8	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.

4.3.2. Samplings and measurements

4.3.2.1. 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.

4.3.2.2. Organ weight and intestinal length measurements

Immediately after the slaughter on day 42, 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 (from gizzard to pancreas-biliary duct), jejunum (from pancreas-biliary ducts to Meckel's diverticulum), and ileum (from Meckel's diverticulum to ileocecal junction) and whole small intestine (as a sum of the three parts) was also measured and expressed relative to body weight (cm/kg BW). The duodenum was defined as the part of the small intestine between the gizzard and the bile duct junction, the jejunum as the section up to Meckel's diverticulum, and the ileum between the Meckel's diverticulum and the ileo-caecal junction.

4.3.2.3. Gut content pH

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).

4.3.2.4. 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. Villus height (VH) was measured as the section from the apical tip of the villus to the villus-crypt junction (crypt mouth), and crypt depth (CD) was measured from the crypt mouth to the top of muscularis mucosae. Villus width (VW) was measured between the brush borders (outer edge of microvilli) of the opposing epithelial cells at the middle of each villus. Muscle layer thickness (MLT) was also measured from the top of the muscularis mucosae to the bottom of muscularis mucosae. For each section, the villus height to crypt ratio (VH/CD) was calculated. Villus surface area (VSA) was also determined according to (Sakamoto et al., 2000) as follows:

$$VSA = 2 \times 3.14\ (VW/2) \times VH$$

4.3.2.5. 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.

4.3.3. 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. applied. In this experiment 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.

5. RESULTS AND DISCUSSION

5.1. First experiment

5.1.1. Production traits

The production performance of broilers is shown in Table 13. No significant difference was observed in FI and BWG during the grower phase among all treatment groups. Unexpectedly, significantly higher BWG was recorded in the finisher phase at the HFSM30 treatment. Feeding the LFSM decreased the weight gain of the birds at 20% numerically, but at 30% significantly, compared with the control. In the grower phase both the HFSM and LFSM diets resulted in significantly higher FCR compared with control. The same tendency was found in the finisher phase, but in this case only the LFSM20 treatment caused significantly higher FCR.

Several studies have been conducted on the effect of feeding SM to broiler chickens (Al-molah and Kloor, 2023; Bilal et al., 2017; Chobanova, 2019; Desai et al., 2018; Ditta and King, 2017; Horvatovic et al., 2015; James and Alagbe, 2022; Mbukwane et al., 2022; Waititu et al., 2018a; Yaqoob et al., 2022). Some findings revealed that dehulled sunflower oilcake can be included in the broiler diet up to 13.5% (Mbukwane et al., 2022), substituting for soybean meal up to 9% (Yaqoob et al., 2022), and a low-fibre, high-protein SM with 45.4% CP replacing soybean meal up to 15%, 25%, and 26.5% at days 10, 24, and day 49 (Gerzilov and Petrov, 2022), respectively, had no negative effect on production traits. The safe inclusion rate for better performance was achieved at 10%, 20%, and 23% in the starter, grower, and finisher phases, respectively. Sunflower meal at 15% and 20% inclusion rate did not affect WG, FI, and FCR, however, at 25% decreased FCR (Bilal et al., 2017). Other findings reported that SM with 36% CP included at 10% had no effect on average BW, daily gain, average FI, and FCR on days 21 and 35 (Attia et al., 2016). Broilers given diets containing 0%, 4%, 8%, and 12% SM had similar BW, FI, and FCR (Sangsoponjit et al., 2017). Based on their effects on the production parameters these research findings were only partly in line with the current study.

Table 13.The effect of dietary treatments on the production traits (mean ± SEM)

Parameters	Treatment	Grower Phase (d 11–24)	Finisher Phase (d 25–38)	Overall Mean (d 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, d—days, 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 ($p < 0.05$).

In our case, the responses depended on the fibre content of SM and on the investigated parameter. High fibre SM, for example, tended to increase the FI of birds but, surprisingly, also resulted in higher BWG at 30%. This positive effect of HFSM was more pronounced in the finisher phase and could be related to the more developed digestive system in the finisher phase, as broilers can tolerate higher fibre at this age. Furthermore, the fibre of HFSM in older chickens can stimulate the gizzard, which might help to reduce viscosity in the small intestine and increase gut motility and the digestion of broiler chickens (Waititu et al., 2018b). Since both FI and BWG increased, HFSM did not modify the FCR in comparison with the control treatment. In our case, LFSM did not affect the FI, however, it impaired the BWG and FCR of chickens. This result was not expected since the diets were isocaloric and isonitrogenous; the reason for the negative effect of LFSM is unknown.

There are also some research outcomes with contradicting results (Al-molah et al., 2023; Amerah et al., 2015; Berwanger et al., 2017b, 2017a; Horvatovic et al., 2015). Replacing soybean meal with dehulled SM in the wheat-corn-soybean based diet fed to non-sexed Rose 308 birds had no effect at up to 30% inclusion level. However, the substitution at 60% (19% of the diet) and 100% (28.2% of the diet) levels caused a linear reduction in BW and FI (Al-molah et al., 2023). Sunflower cake was fed to Cobb 500 birds at a rate of 0%, 5%, 10%, 15%, and 20% both at the age of 1 to 21 days (Berwanger et al., 2017a, 2017b) and 22 to 42 days (Berwanger et al., 2017b). Their findings revealed that at 15% and 20% inclusions the final BW, WG, and FI linearly decreased with increasing levels of sunflower cake. There were slight differences in birds' type, age, sex, sources of energy, feed ingredients used for feed formulation, the type of SM used, and inclusion rates between the above-mentioned studies and our trial, which could be the reason for the contradicting results. In addition, the higher amount of sunflower oil added to the HFSM- and LFSM-containing diet might also affect the production traits. Findings revealed sunflower oil is rich in flavonoids an antioxidant that reduces the oxidative stress, improves immune function, stimulates intestinal tract functions, enhances digestive secretions, nutrient absorption, and metabolism, thereby improving growth due to the bioactive compounds in the oil that support intestinal health, boost digestion, and scavenge free radicals (James and Alagbe, 2022; Oluwafemi et al., 2021). To evaluate the fibre and fat effects separately warrants further investigation.

5.1.2. Effects of dietary treatments on carcass composition

Table 14. shows that the SM treatments did not affect the relative carcass, breast meat, and thigh weights. The abdominal fat percentage, however, increased when the LFSM-containing diets were fed. The difference between the LFSM20 and HFSM30 groups was significant. The abdominal fat percentage of birds fed LFSM20 was 54.24% higher than the birds fed HFSM30.

The SM treatments did not show a significant difference in carcass yield and carcass cuts (deboned breast meat and thighs). Both LFSM diets resulted in higher abdominal fat. However, significantly higher abdominal fat was observed only between the groups fed LFSM20 than 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.

Table 14. The effect of dietary treatments on carcass composition (day 41; mean ± SEM)

Treatments	**Carcass (%)**	**Breast Meat (%)**	**Thigh (%)**	**Abdominal Fat (%)**
C	67.89 ± 0.76	23.08 ± 0.66	19.42 ± 0.47	0.83 ± 0.11 ^{ab}
HFSM20	67.19 ± 0.63	22.43 ± 0.81	19.51 ± 0.54	0.70 ± 0.10 ^{ab}
HFSM30	66.96 ± 0.61	21.66 ± 0.57	19.32 ± 0.56	0.64 ± 0.11 ^{b}
LFSM20	63.70 ± 2.93	21.91 ± 0.64	18.71 ± 0.41	1.18 ± 0.19 ^{a}
LFSM30	65.13 ± 1.48	20.08 ± 0.95	18.47 ± 0.35	0.95 ± 0.08 ^{ab}
p-value	0.289	0.083	0.479	0.030

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} means with different superscripts in the same column are significantly different ($p < 0.05$).

Berwanger et al. (2017b) also reported that sunflower cake does not significantly affect the carcass cuts and abdominal fat percentage at inclusion levels up to 20%, and carcass yield up to 15%. Similarly, SM up to 20% did not have a significant difference in carcass weight and carcass cuts (Aziz and Rashid, 2023); likewise, the inclusion of SM at 4%, 8%, and 12% did not affect carcass characteristics on day 42 (Sangsoponjit et al., 2017). Sunflower meal at 0%, 5%, 6%, 8%, and 10% did not affect jejunal digesta viscosity, carcass characteristics, breast meat yield, and abdominal fat (Amerah et al., 2015). Sunflower inclusion at 6% and 8% in the growers' stage, and at 10% and 16% during the finisher stage had no effect on carcass traits (Horvatovic et al., 2015). When SM was included up to 10% in the broiler diets, no difference was observed in dressing percentage (Attia et al., 2016). Sunflower cake failed to affect carcass yield and abdominal fat up to 10% inclusion and even did not affect breast and thigh-plus-drumstick when increased to 20% compared with the control (Berwanger et al., 2017a).

These findings align with the present study, where the inclusion of HFSM and LFSM up to 30% had no effect on carcass weight, breast fillet, thigh-plus-leg weight, or abdominal fat percentages compared to the control diet. However, it is important to note that, though all these findings agree with the present study, their inclusion rates were less than 30%. Therefore, 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.

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

The treatments resulted in nutrient dependent changes in the digestibility. Low fibre SM at both inclusion rates improved the absorption of crude fat, at $p < 0.001$, during the grower phase (Table 15). On the other hand, all SM-containing diets decreased the digestibility of starch in comparison with the control. The lowest starch digestion belonged to the HFSM30 group. The treatments affected AME in line with the digestibility of fats. The LFSM diet at 30% inclusion rate increased AMEn by 0.7 KJ/g. The nitrogen retention of chickens was affected only by the treatment LFSM30 during the grower phase. This diet resulted in significantly higher N retention in comparison with the control and HFSM groups. During the finisher phase, at day 41, the highest and lowest fat digestibility was recorded in the case of the LFSM30 and HFSM20 diets, respectively. The starch digestion was also affected negatively by SM diets at this age. The differences were significant for the HFSM30 and LFSM30 treatments. The N retention of chickens did not change in the older birds.

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. However, LFSM significantly decreased starch digestibility (day 27) and only at 30% (day 41). The N retention of birds increased in the LFSM group only during the grower phase.

The traditional HFSM failed to modify fat digestibility, AMEn and N retention at day 27, however, decreased the digestion of starch. During the finisher phase 20%, HFSM decreased fat digestibility and decreased starch digestion at both inclusion rates but did not modify AMEn and N retention in comparison with the control treatment. 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). It is well known, that if the gizzard does not work properly and the chicken over consume the feed, manly starch digestibility is impaired. So, it could be the other explanation, starch digestion is more sensitive to the change in the passage rate**.** However, the improvement over the control is hard to explain.

Broiler chickens were fed on three types of SMs that vary in the degree of dehulling, resulting in CP contents of 37.4%, 40%, and 42.5% and corresponding CF contents of 21.7%, 20.2%, and 17.2%, respectively. These meals were included in the diet at a rate of 20% from day 8 to day 21. No significant differences in AME were observed between the first two SM types; however, the third high-protein, low-fibre SM significantly increased AME (Karkelanov et al., 2020).

Table 15. 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.

These results can be compared with our HFSM and LFSM results at day 27, where HFSM did not affect AME digestibility, whereas LFSM significantly improved AME digestibility. Sunflower meal at 15%, 20%, and 25% levels in the broiler diet linearly improved crude fibre digestibility but failed to affect DM, CP, and fat digestibility on day 41 and 42 (Bilal et al., 2017). The enhanced CF digestibility could be linked to the increased gut motility, increased release of energy and nutrients, and the breakdown of fibre during digestion.

5.1.4. Ileal amino acid digestibility on day 27

The apparent ileal AA digestibility values at day 27 are shown in Table 16. 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. Leucine 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 16. Apparent ileal amino acid digestibility at day 27 (mean ± SEM)

AA	**C**	**HFSM20**	**HFSM30**	**LFSM20**	**LFSM30**	**P-value**
ARG	80.08 ± 0.76^{c}	84.68 ± 0.31^{ab}	84.50 ± 1.10^{b}	87.64 ± 1.12^{ab}	88.04 ± 0.69^{a}	<0.001
HIS	77.79 ± 0.75^{b}	78.59 ± 0.42^{b}	77.24 ± 0.63^{b}	81.50 ± 1.27^{ab}	81.33 ± 0.66^{a}	0.003
ILE	76.83 ± 0.60^{b}	77.79 ± 0.41^{b}	82.52 ± 0.47^{a}	83.51 ± 1.50^{a}	83.05 ± 0.82^{a}	<0.001
LEU	86.20 ± 0.37^{a}	83.75 ± 0.46^{b}	80.59 ± 0.48^{c}	84.25 ± 1.29^{abc}	85.08 ± 0.56^{ab}	<0.001
LYS	72.27 ± 1.27^{b}	74.92 ± 0.71^{ab}	73.96 ± 1.19^{ab}	77.25 ± 1.99^{a}	77.39 ± 1.12^{a}	0.044
MET	84.79 ± 0.61^{bc}	83.54 ± 0.30^{c}	84.29 ± 0.51^{c}	87.28 ± 0.98^{ab}	88.42 ± 0.53^{a}	<0.001
PHE	83.90 ± 0.65^{ab}	82.88 ± 0.42^{ab}	82.44 ± 0.57^{b}	85.02 ± 1.18^{ab}	85.49 ± 0.57^{a}	0.022
THR	58.28 ± 0.98^{b}	67.00 ± 1.03^{a}	69.95 ± 2.11^{a}	70.27 ± 1.71^{a}	69.60 ± 1.31^{a}	<0.001
VAL	74.61 ± 0.69^{b}	79.24 ± 1.40^{a}	78.11 ± 1.08^{ab}	82.08 ± 1.36^{a}	81.88 ± 0.89^{a}	<0.001
TYR	71.76 ± 1.50^{c}	75.43 ± 0.96^{bc}	77.32 ± 0.71^{b}	83.22 ± 1.08^{a}	82.89 ± 0.82^{a}	<0.001
CYS	74.69 ± 1.05^{bc}	74.36 ± 0.81^{c}	78.64 ± 1.0^{a}	78.07 ± 1.00^{ab}	77.65 ± 0.56^{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, 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 ($p < 0.05$).

5.1.5. Amino acid digestibility on day 41

5.1.5.1. Amino acid digestibility in the distal jejunum

The apparent amino acids digestibility values in the distal jejunum at day 41 are presented in Table 17. Compared with the control diet, feeding HFSM at both inclusion rates improved the digestibility of THR and TYR digestibility. High fibre SM at 20% level 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 17. Apparent amino acids digestibility in the distal jejunum at day 41 (mean ± SE)

AA	**C**	**HFSM20**	**HFSM30**	**LFSM20**	**LFSM30**	**P-value**
ARG	70.02 ± 1.75^{b}	72.34 ± 0.77^{ab}	75.63 ± 1.07^{a}	74.94 ± 1.20^{ab}	73.65 ± 0.98^{ab}	0.019
HIS	63.68 ± 1.58	68.74 ± 1.50	67.02 ± 1.58	65.53 ± 2.10	67.54 ± 1.41	0.226
ILE	64.38 ± 1.62^{b}	71.38 ± 0.77^{a}	69.54 ± 1.50^{ab}	64.27 ± 1.38^{b}	65.25 ± 0.80^{b}	<0.001
LEU	73.66 ± 1.32	72.95 ± 1.33	71.97 ± 0.98	71.69 ± 1.59	69.68 ± 1.30	0.237
LYS	56.70 ± 2.62	61.22 ± 1.25	61.09 ± 1.31	62.83 ± 2.03	63.54 ± 1.42	0.085
MET	67.15 ± 1.76^{b}	73.60 ± 1.18^{ab}	74.73 ± 1.26^{a}	74.54 ± 1.28^{a}	74.64 ±0.79^{a}	0.029
PHE	72.85 ± 1.31	73.59 ± 0.61	72.30 ± 1.18	69.34 ± 1.88	72.48 ± 1.06	0.227
THR	44.87 ± 2.34^{c}	58.65 ± 1.94^{a}	54.57 ± 1.79^{ab}	55.90 ± 2.13^{ab}	50.30 ± 1.61^{bc}	<0.001
VAL	63.66 ± 2.14	68.25 ± 1.66	64.80 ± 1.21	66.21 ± 1.55	65.80 ±1.32	0.366
CYS	56.74 ± 2.32	62.55 ± 2.03	62.11 ± 1.84	60.60 ± 2.27	62.84 ±1.26	0.161
TYR	58.79 ± 0.97^{b}	69.66 ± 1.56^{a}	65.45 ± 0.16^{a}	63.62 ± 2.29^{ab}	68.52 ± 1.35^{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, 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 ($p < 0.05$).

5.1.5.2 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 18. Both HFSM treatments increased the digestibility of LYS, THR, and TYR, while decreased that of LEU. HFSM30 further increased the digestibility of ARG and ILE.

The HFSM30 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 ($p < 0.001$) except that of LEU.

Table 18. Apparent amino acid digestibility in the proximal ileum at day 41 (mean ± SEM)

AA	C	HFSM20	HFSM30	LFSM20	LFSM30	P-value
ARG	80.44 ± 0.56^{c}	82.83 ± 0.71^{bc}	83.66 ± 0.93^{b}	88.76 ± 0.38^{a}	87.87 ± 0.49^{a}	<0.001
HIS	75.25 ± 0.89^{b}	76.77 ± 0.34^{b}	77.37 ± 1.01^{b}	82.43 ± 0.70^{a}	82.48 ± 0.49^{a}	<0.001
ILE	77.34 ± 0.72^{d}	79.32 ± 0.08^{cd}	80.55 ± 0.67^{bc}	82.76 ± 0.49^{ab}	83.55 ± 0.48^{a}	<0.001
LEU	85.15 ± 0.36^{a}	82.10 ± 0.34^{b}	81.64 ± 0.76^{b}	84.85 ± 0.40^{a}	85.39 ± 0.30^{a}	<0.001
LYS	68.47 ± 0.29^{c}	72.71 ± 0.61^{b}	74.15 ± 1.20^{ab}	77.31 ± 0.38^{a}	77.40 ± 0.54^{a}	<0.001
MET	84.24 ± 0.41^{b}	84.00 ± 0.34^{b}	85.06 ± 0.47^{b}	88.36 ± 0.52^{a}	88.47 ± 0.44^{a}	<0.001
PHE	82.53 ± 0.63^{b}	82.58 ± 0.43^{b}	82.55 ± 0.74^{b}	85.19 ± 0.44^{a}	85.36 ± 0.47^{a}	<0.001
THR	58.04 ± 0.61^{b}	67.99 ± 0.68^{a}	65.37 ± 1.42^{a}	70.36 ± 1.42^{a}	69.24 ± 0.54^{a}	<0.001
VAL	75.16 ± 1.18^{b}	77.74 ± 0.73^{b}	78.37 ± 1.19^{ab}	81.92 ± 0.48^{a}	81.56 ± 0.39^{a}	<0.001
TYR	69.56 ± 1.05^{c}	77.18 ± 0.78^{b}	78.70 ± 0.60^{b}	82.70 ± 0.67^{a}	82.36 ± 0.92^{a}	<0.001
CYS	72.70 ± 0.64^{b}	73.77 ± 0.75^{b}	74.68 ± 0.40^{b}	79.27 ± 1.12^{a}	77.96 ± 0.43^{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, 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).

5.1.5.3 Amino acid digestibility in the distal ileum

The results are shown in Table 19. Mainly the same amino acids were affected as in the previous ileal part, ARG, THR, VAL, TYR, LEU (HFSM20, HFSM30), LYS (HFSM20) all amino acids except LEU (LFSM20, LFSM30). Again, the effect of HFSM on leucine digestibility was negative while unaffected by LFSM.

Table 19. Apparent amino acids digestibility in the distal ileum at day 41 (mean ± SEM)

AA	C	HFSM20	HFSM30	LFSM20	LFSM30	P-value
ARG	81.30 ± 0.38^{c}	84.05 ± 0.67^{b}	86.14 ± 0.42 ^{ab}	87.73 ± 0.55^{a}	87.31 ± 0.46^{a}	<0.001
HIS	77.91 ± 0.40^{b}	79.23 ± 0.70^{ab}	77.95 ± 0.64^{b}	81.46 ± 0.69^{a}	81.64 ± 0.60^{a}	<0.001
ILE	80.87 ± 0.43^{b}	79.69 ± 0.91^{b}	79.49 ± 0.70^{b}	83.81 ± 0.58^{a}	84.02 ± 0.49^{a}	<0.001
LEU	87.08 ± 0.47^{a}	83.49 ± 0.45^{bc}	82.38 ± 0.54^{c}	85.40 ± 0.48^{a}	85.27 ± 0.37^{ab}	<0.001
LYS	71.27 ± 0.61^{b}	75.86 ± 0.92^{a}	72.45 ± 0.97^{b}	76.56 ± 0.76^{a}	76.32 ± 0.60^{a}	<0.001
MET	85.20 ± 0.41^{b}	84.68 ± 0.68^{b}	85.67 ± 0.38^{b}	89.67 ± 0.34^{a}	88.19 ± 0.20^{a}	<0.001
PHE	83.73 ± 0.23^{b}	84.11 ± 0.61^{b}	82.40 ± 0.51^{b}	86.08 ± 0.50^{a}	86.40 ± 0.22^{a}	<0.001
THR	58.80 ± 1.58^{b}	67.85 ± 1.60^{a}	66.07 ± 1.00^{a}	71.25 ± 1.27^{a}	68.25 ± 0.79^{a}	<0.001
VAL	77.18 ± 0.35^{d}	80.54 ± 0.58b^{c}	79.15 ± 0.44^{c}	83.44 ± 0.59^{a}	82.20 ± 0.21^{ab}	<0.001
TYR	71.87 ± 0.66^{c}	81.29 ± 0.62^{ab}	79.72 ± 0.58^{b}	83.22 ± 0.53^{a}	81.28 ± 1.08^{ab}	<0.001
CYS	73.28 ± 0.70^{c}	74.22 ± 0.99^{c}	75.22 ± 0.94b^{c}	81.04 ± 0.75^{a}	77.72 ± 0.78^{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, 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).

The proximate analysis of 238 SM samples after solvent extraction were crude fiber 18.8% (6.53%–28.65%), ADF 22.5% (8.49%–37.69%), and NDF 32.15% (13.74%–45.59%). The mean CP content of 739 samples was 32.2% (21.0%–51.9%) (Wiltafsky et al., 2016). The protein content of a high protein SM was 48.2%. But the most abundant and least abundant amino acids were arginine (3.7%) and methionine (0.75%), respectively (Waititu et al., 2018a). The highest AA content of two tested high protein SM was arginine 6.4 g/100g in SM40+ and 7.9 g/100g protein in SM47+ and the lowest was isoleucine 1.91 g/kg in SM 40+ and tyrosine 2.33 g/kg protein in SM47+ (Chobanova and Penkov, 2021). Compared with our study, the protein content of HFSM (38.4%) and LFSM (43.8%) was a little lower, however, in the current study the most abundant amino acid was methionine (7.92%-HFSM and 9.38%-LFSM) and the least abundant amino acid was arginine (0.67%-HFSM, and 0.70%-LFSM) which was against their results (Table 8). Our result agrees with the fact that SM is reach in sulphur containing amino acids (Chobanova, 2019). 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.

On day 27, the difference in the ileal AA digestibility between the HFSM and control groups was significant. HFSM increased the digestibility of ARG, ILE, THR, TYR and CYS. LFSM as a whole, and LFSM30 in particular, increased digestibility of most of the amino acids compared with the control (ARG, HIS, ILE, LYS, MET, THR, VAL, TYR) (Table 16). On day 41, in the proximal ileum**,** both HFSM treatments increased the digestibility of LYS, THR, and TYR. Surprisingly, the inclusion of LFSM at 20% and 30% significantly enhanced the digestibility of all amino acids (Table 18). A similar result was observed in the distal ileum (Table 19). In both ileal parts HFSM decreased leucine digestibility while unaffected in the jejunal part. Feeding LFSM did not affect leucine digestibility in all parts of the intestine.

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., 2024a). 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. 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.

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 17), and the jejunal AA digestibility relative to distal ileal digestibility was also higher when HFSM was fed (Table 22). This indicates that in the jejunum the gizzard stimulatory effect of SM dominated. In the ileum, however, the mucosa erosion and the other endogenous protein secretions negatively affected the apparent digestibility of amino acids. From amino acid digestion point of view around 5-6% crude fibre seems to be upper limit, but in this trial the production was not in line with the amino acid digestibility coefficients.

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 leucine digestibility remained unaffected after the animals were fed with LFSM. Therefore, 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. Already the use of high protein-low fiber SM in poultry diets is widespread (Waititu et al., 2018b) and can be applied to replace soybean meal.

Although the diets were isocaloric and isonitrogenous, the digestion of amino acids could have been affected by the differences in the starch and fat content of the diets.

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. Our findings, HFSM decreased villus hight and increased the concentration of isovaleric acid in the caeca, support this assumption and indicates broiler chickens have an optimal structural fibre interval, that already stimulates the gizzard but results only limited increase in epithelial erosion and passage rate. 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.

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

The two-factorial evaluation results are shown in Table 20. 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, but except VAL and TYR no difference was observed between the proximal and distal ileal digestibility. The feed and intestinal part interaction was significant for most amino acids.

The intestinal part affected digestibility of amino acids among the dietary treatments (Table 20). Jejunal AA digestibility was lower than ileal AA digestibility. AA digestibility was similar in the proximal and distal ileum, except for valine and tyrosine which were higher in the distal ileum. LFSM increased the digestibility of all AAs, while leucine was not affected. HFSM also increased the digestibility of all AAs, but decreased leucine digestibility. It was clear in these results, that AA digestibility in the finisher phase was higher due to the fibre contained in SM meal.

Table 20. Dietary treatments and intestinal segments effects on the digestibility of amino acids at day 41 (%)

Feed (F)	**Intestinal part (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 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. The highest AA digestibility was recorded for arginine or methionine, whereas the lowest was recorded for threonine in the three sections of the small intestine (Table 20).

True AA digestibility of two high protein SMs was tested. Arginine demonstrated the highest AA digestibility, whereas threonine showed the lowest in both intact and caecectomized birds (Chobanova and Penkov, 2021). A comparison of ileal and excreta AA digestibility of SM showed that methionine and threonine exhibited the highest and lowest ileal AA digestibility, respectively (Ravindran et al., 1999). These findings were in line with the ileal amino acid digestibility results observed in the present study. Most of the AAs showed a significantly higher interaction effect with the intestinal segments, the reason for it was that LFSM30 or HFSM30 resulted in lower distal ileal digestibility compared with the proximal ileal coefficients. The standard ileal AA digestibility of solvent extracted SM was lysine 87%, methionine 92%, cystine 80%, threonine 82%, tryptophan 87%, arginine 93%, isoleucine 89%, leucine 88%, valine 87% and phenylalanine 90% (Wiltafsky et al., 2016). The ileal amino acid digestibility of SM in our experiment was slightly lower. AA digestibility is influenced by multiple factors related to both diet composition and animal characteristics. Evaluation of dietary protein is more precise when expressed as digestible amino acids instead of total amino acids. AAs that escape digestion and absorption in the small intestine may undergo microbial fermentation in the caeca and colon and therefore are not available to the host animal (Blok et al., 2017). For this reason, ileal AA digestibility is more precise indicator of AA availability to the animal than digestibility determined over the entire digestive tract. The backward peristaltic movement of the hindgut can also contribute microbial protein to the terminal ileum. In the current study, absorption of the digested AAs was increased when the feed moves from the jejunum to the distal ileum. Ileal AA digestibility was significantly higher than distal jejunum AA digestibility. However, there were no difference between proximal and distal ileum AA digestibility, an indication no further digestion of AA happened in the distal ileal part and also no significant amount of microbial protein was contributed via the backward peristaltic movement of the hind gut. In our previous study, the same amount and type of SM was fed to the same bird type and age groups of the current study. These results revealed there were no significant difference in SCFAs among all dietary treatments and inclusion levels. The probable reason could be the particle size of the SM diets that no fine and soluble particles were entered the ceca (Tewelde et al., 2026a). It was also reported that the reason for low NSP degradation is the short retention time (7 to 15 hours) in the main fermentation cite of chickens, the ceca and only liquid and fibre sources with small particle size (< 0.2 mm) can pass and enter the ceca (Lannuzel et al., 2022)

5.1.7. 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 21. In this case the AA digestibility values were calculated only from the total ileal contents of both age categories. Apart from LEU, LFSM enhanced the ileal digestibility of all AAs in comparison with the control. However, HFSM decreased the absorption rate of LEU and resulted increase in the case of ARG, ILE, LYS, THR, VAL and TYR.

The age of chickens resulted in significant difference only in the case of MET and TYR. The digestibility of these two amino acids was 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).

The standard ilea AA digestibility of SM was lysine (87%), methionine (92%), cystine (80%), M+C (87%), threonine (82%), tryptophane (87%), arginine (93%), isoleucine (89%), leucine (88%), valine (87%), histidine (88%) and phenylalanine (90%) (Lemme et al., 2004). Dietary fiber has both negative and positive effects based on its solubility and inclusion levels (Vivares et al., 2025). Birds age also have 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).

Table 21**.** Ileal amino acid digestibility of amino acids at 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	0.35
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 ($p < 0.05$).

Extensive hull removal of safflower seed makes it suitable to be incorporated in broilers diet as this process reduces the fiber content and slightly increases the amino acid concentration (Farran et al., 2010). In our previous investigation HFSM and LFSM both at 20% and 30% were fed in the grower and finisher phases. Birds fed HFSM at 30% level had a significantly lower viscosity compared with the other treatments, because the IDF content of HFSM was very high. Furthermore, viscosity was higher on day 42 compared with day 27, showing the age effect (Tewelde et al., 2026a), therefore, low fiber SM should be used for younger age categories (Tewelde et al., 2026b). Sunflower meal with 35% CP and 18.5% CF was also fed to pullets at 0%, 10%, 20%, and 30%. Sunflower meal increased the digestibility of AAs such as threonine, valine, lysine, arginine, and tyrosine. However, impaired the digestibility of leucine (Such et al., 2024a). Their result was in line with the effect of HFSM and LFSM in the current study (Table 21). In contrast to the current study, a corn-soybean meal basal diet replaced by 30% high protein SM was fed to broilers from day 14 to 21. The highest AA digestibility was observed for arginine (89%) and the lowest for histidine (65%) (Waititu et al., 2018a). The source of contrasting results could be the age of the birds.

5.1.8. 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 2. 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.

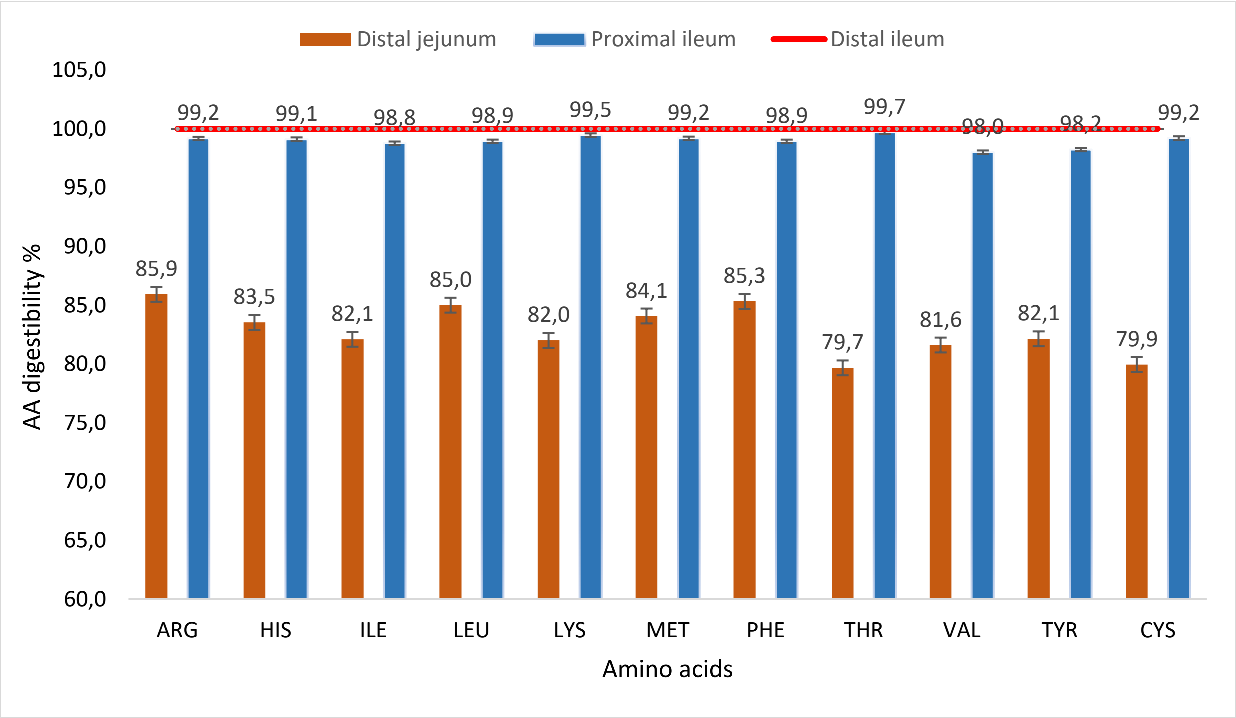

Figure 2. Amino acid disappearance in the distal jejunal and proximal ileal parts relative to the distal ileal AA disappearance

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

5.1.9. Amino acid absorption dynamics from the distal jejunum

Dietary treatment effects on the distal jejunal relative AA digestibility are presented in Table 22. 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 ($p < 0.001$). The digestibility of PHE, TYR, and CYS was also affected by the diets, but the differences in these AAs were significant only between the SM treatments.

Table 22. 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 ± 2.2	86.3 ± 1.1	87.8 ± 1.3	84.7 ± 1.4	84.4 ± 1.3	0.469
Histidine	81.5 ± 2.5	87.2 ± 2.2	86.0 ± 2.1	79.6 ± 2.5	82.8 ± 1.9	0.137
Isoleucine	79.4 ± 2.6^{b}	90.3 ± 1.4^{a}	87.5 ± 1.8^{a}	76.2 ± 1.8^{b}	77.7 ± 1.1^{b}	<0.001
Leucine	84.1 ± 1.9	87.6 ± 1.7	87.4 ± 1.3	83.4 ± 1.9	81.7 ± 1.5	0.052
Lysine	78.0 ± 3.9	81.2 ± 1.9	84.4 ± 2.2	81.1 ± 2.9	83.3 ± 2.2	0.484
Methionine	78.1 ± 2.4^{c}	87.2 ± 1.4^{a}	87.2 ± 1.4^{a}	82.7 ± 1.5^{ab}	84.6 ± 0.8^{a}	0.001
Phenylalanine	86.3 ± 1.6^{ab}	87.7 ± 1.1^{a}	87.8 ± 1.7^{a}	79.9 ± 2.1^{b}	83.9 ± 1.2^{ab}	0.008
Threonine	74.8 ± 4.6	84.7 ± 2.0	82.7 ± 3.0	76.6 ± 3.1	73.8 ± 2.6	0.063
Valine	81.8 ± 3.1	84.9 ± 1.5	81.9 ± 1.4	78.7 ± 2.0	80.1 ± 1.6	0.303
Tyrosine	81.4 ± 1.7^{ab}	85.8 ± 1.8^{a}	82.8 ± 0.8^{ab}	76.1 ± 2.8^{b}	84.5 ± 2.3^{a}	0.019
Cystine	75.8 ± 3.5^{ab}	84.7 ± 2.2^{a}	82.6 ± 2.3^{ab}	73.9 ± 2.6^{b}	80.9 ± 1.7^{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 ($p < 0.05$).

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. Digestion and absorption of nutrients in the gastrointestinal tract (GIT) is a two-stage process, involving enzymatic breakdown and transport of products across the intestinal epithelium (Ravindran and Abdollahi, 2021). Relative to the distal halves, the effect of fiber on the AA digestibility was evident. Specifically, AA digestibility in the jejunum was higher in the HFSM treatments than in the C diet and LFSM treatments. Isoleucine digestibility when HFSM20 was fed was the highest relative to distal ileum AA digestibility of the dietary treatments (Table 22). 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).

5.1.10. 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 23). 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. Since SM is relatively rich in soluble fibre, it presents a potential constraint. 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). It can happen in the barn, during manure storage, or while spreading the manure in the field. 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 23. The dry matter content (%) and the different nitrogen forms of the excreta (mg/g 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 ($p < 0.05$)

However, protein and AA retention decrease with increasing age of birds and generally is maximum during the early growth. In broilers, N-retention increases until 28 days of age, although when expressed as a percentage of N-intake, there was decline over time, especially after day 28 (Lopez and Leeson, 2007; Vilela et al., 2020). This age-related decline may partly explain why the mechanism was not observed during the finishers phase.

5.1.11. 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 (Table 24). Interestingly, feeding HFSM at 30% resulted in the lowest viscosity ($p < 0.001$) at both age groups. The age and feed interaction effect on digesta viscosity showed that growers had a significantly lower viscosity ($p = 0.005$) an indication that viscosity increases with age.

An interesting result of this trial was that the HFSM decreased the viscosity in the jejunal content. The difference in the HFSM30 treatment was significant. Other findings reported that the addition of SM into the broiler-grower diets at a rate of 6% and 8% and into the finisher diets at 10% and 16% significantly increased jejunal and ileal viscosity (Horvatovic et al., 2015). This result is against our finding. However, Bilal et al. (2017) found that SM at 25% inclusion rate modify fibre digestibility and gut motility. The inclusion of SM at 16% and 20% during the grower and finisher phases, respectively, reduced jejunal viscosity (Such et al., 2024b), which agrees with the current findings. Therefore, 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.

Table 24. Feed and age interaction effect of the jejunal digesta viscosity (cP) (mean ± SEM)

Age	**Feed**	**Viscosity**
Day 27	C	4.0 ± 0.12 ^{a}
	HFSM20	3.7 ± 0.17 ^{ab}
	HFSM30	3.2 ± 0.07 ^{b}
	LFSM20	3.7 ± 0.11 ^{ab}
	LFSM30	3.5 ± 0.17 ^{ab}
p-value		<0.001
Day 41	C	4.0 ± 0.08 ^{ab}
	HFSM20	4.2 ± 0.16 ^{a}
	HFSM30	3.4 ± 0.03 ^{b}
	LFSM20	4.4 ± 0.14 ^{a}
	LFSM30	4.3 ± 0.17 ^{a}
p-value		<0.001
Feed	C	4. 0 ± 0.09 ^{a}
	HFSM20	4.0 ± 0. 09 ^{a}
	HFSM30	3.3 ± 0. 09 ^{b}
	LFSM20	4.0 ± 0. 09 ^{a}
	LFSM30	3.9 ± 0.11 ^{a}
Age	Day 27	3.6 ± 0.06 ^{b}
	Day 41	4.1 ± 0.06 ^{a}
	SEM	0.06
p-values	Feed	<0.001
	Age	<0.001
	Feed X age	0.005

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} means with different letters are significantly different ($p < 0.05$)

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 the age. The average viscosity at day 41 was significantly 13.89% higher than that at day 27. The reason for the age-related difference could be that the birds in the finisher phase consumed large amount of feed 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. Inclusion of 6% SM during the starter and 10% during the grower or finisher phases had no effect on jejunal digesta viscosity. However, feeding 16% and 20% SM to pullets and layers, respectively (Such et al., 2024b), as well as inclusion of dehulled SM up to 13.5% measured at 35 days, reduced jejunal digesta viscosity (Mbukwane et al., 2022), which is not in line with our LFSM results. The SCFA content of the caeca was not influenced by the SM treatments (Table 25).

Table 25. The effect of dietary treatments on the caecal short-chain fatty acids of broiler chicks (μmol/g)

Age	**Treatments**	**Acetic Acid**	**Propionic Acid**	**Isobutyric Acid**	**Butyric Acid**	**Isovaleric Acid**	**Valeric Acid**
Day 27	C	2.28 ± 0.19	0.52 ±0.07	0.07 ± 0.01	0.50 ± 0.07	0.07 ± 0.02	0.07 ± 0.02
	HFSM20	2.16 ± 0.24	0.51 ± 0.06	0.06 ± 0.01	0.43 ± 0.06	0.08 ± 0.02	0.08 ± 0.01
	HFSM30	2.16 ± 0.25	0.49 ± 0.06	0.07 ± 0.01	0.46 ± 0.06	0.08 ± 0.02	0.07 ± 0.01
	LFSM20	2.43 ± 0.24	0.50 ± 0.06	0.06 ± 0.01	0.50 ± 0.07	0.06 ± 0.01	0.09 ± 0.01
	LFSM30	2.33 ± 0.24	0.43 ± 0.06	0.06 ±0.01	0.57 ± 0.07	0.07 ± 0.01	0.08 ± 0.01
	p-value	0.918	0.888	0.974	0.692	0.816	0.938
Day 41	C	2.23 ± 0.23	0.50 ± 0.06	0.06 ± 0.01	0.40 ± 0.04	0.07 ± 0.01	0.06 ± 0.01
	HFSM20	2.47 ± 0.20	0.52 ± 0.06	0.06 ± 0.01	0.47 ± 0.06	0.07 ± 0.01	0.07 ± 0.02
	HFSM30	2.13 ± 0.24	0.50 ± 0.09	0.04 ± 0.01	0.46 ± 0.07	0.06 ± 0.01	0.05 ± 0.01
	LFSM20	2.26 ± 0.26	0.48 ± 0.06	0.06 ± 0.01	0.51 ± 0.06	0.07 ± 0.01	0.06 ± 0.01
	LFSM30	2.30 ± 0.26	0.49 ± 0.06	0.06 ± 0.01	0.48 ± 0.05	0.07 ± 0.01	0.07 ± 0.01
	p-value	0.903	0.991	0.488	0.724	0.974	0.553

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.

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.

The results show that the fibre content of the extracted SMs has a significant effect on the production traits, nutrient digestibility, the excretion of the different N compounds, and the gut viscosity of broiler chickens. The SM effects are also age dependent, which should be considered in the diet formulation.

5.2. Second experiment

5.2.1. Production traits

The production performance of the broilers is shown in Table 26. 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 improved BWG, and 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. 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. This partly agrees with our previous results, in which feeding high and low fiber SM at 20% and 30% levels to broiler grower (day 24) and finishers (day 38) did not affect FI and BWG on day 24, but high fiber SM at 30% improved BWG on day 38 (Tewelde et al., 2026a).

Sunflower seed cake was fed to broilers from day 22 to day 42 at inclusion levels of 0%, 5%, 10%, 15%, and 20%, with and without an enzyme complex composed of pectinase, protease, phytase, glucanase, xylanase, cellulase and amylase at 4000, 700, 300, 200, 100, 40 and 30 U/g respectively, applied at 0.02% of the total diet.

Table 26. The effects of dietary treatments on the production traits (mean ± SEM)

	Feed (F)	Phytase (P)	FI (g)	BWG (g)	FCR (g/g)
Grower phase	C	-	1197.8	856.3	1.40
		+	1185.8	853.9	1.38
(Day 11–24)	SM	-	1188.7	897.6	1.33
		+	1190.0	880.2	1.37
Finisher phase	C	-	1639.6	974.8	1.67
		+	1743.0	1059.5	1.63
(Days 25–36)	SM	-	1710.3	1168.6	1.47
		+	1698.9	1148.6	1.50
Overall mean	C	-	2837.4	1831.1	1.57
		+	2928.8	1913.4	1.53
(Days 0–36)	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	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}
(Days 0–36)	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
		SEM	8.7	8.9	0.01
P-values	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.

The findings showed enzyme addition had no effect on the production traits (FI, BWG and FCR), which agrees with the findings of this experiment. In contrast, production traits linearly worsened as sunflower cake inclusion increased. Final body weight, BWG, FCR were worse in birds fed sunflower cake at 15% and 20% compared to those fed the control diet (Berwanger et al., 2017a). Their contrasting result could be due to the high fibre content of sunflower cake and its low protein content (19.5% CP) compared to the SM used in our study (35.5% CP, and 19% CF).

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

Another study examined the effects of sorghum-based diets on the interactions between xylanase, protease, and superdosing phytase in broiler performance, carcass yield, and digesta transit time. According to the results, superdosing phytase increased BW and FI at 42 days of age, while xylanase improved FCR. Although, in conclusion, the beneficial effects of xylanase, protease, and superdosing phytase on broiler performance were not additive. In their opinion, this limitation was likely not related to the lack of efficacy of any one of the individual enzymes, but rather to a limitation in the bird's ability to respond additively to successive additions of enzymes (dos Santos et al., 2017).

On a study SM (0%, 10%, 20%) with two levels of NSPase supplementation (0 or 100 g/ton feed) replaced soybean for Ross–308 broiler chicks. Diets were formulated for starter (1—10 days), grower (11—21 days), and finisher (22—35 days). Sunflower meal up to 20% and NSPase supplementation had no effect on FI, BWG, and FCR on days 21 and 35 (Munawar et al., 2025). Similarly, feeding SM at 20% on day 24 and phytase supplementation resulted in no difference in the current study. But 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).

The effect of including SM at 15%, 20% and 25% with and without NSPase enzyme supplementation was evaluated. Feeding SM up to 20%, regardless of enzyme addition, improved BWG, FI, and FCR. However, increasing SM up to 25% decreased FCR. Importantly, this problem was solved with NSPase supplementation (Bilal et al., 2017). The effect of the enzyme contrasts with our result. Soybean meal replaced with SM (0%, 25%, 50%, and 75%) along with two levels of Avizyme supplementation (0% or 0.1g/kg diet) showed that replacing soybean with SM up to 50% increased BWG and FCR during 22 to 42 and 7 to 42 days compared to control diet (Alagawany et al., 2018). Soybean meal replaced with high-protein SM at levels 25%, 50%, 75%, and 100% from days 0 to 35. Increasing SM levels linearly decreased both FI and BWG. On their second experiment the control diet compared with high protein SM at 75% inclusion level with and without two types of multienzymes were investigated. On day 21, still feeding 75% SM reduced BWG and gain to feed ratio, however, the enzymes alleviate BWG and gain to feed ratio (Waititu et al., 2018b) as a result no difference was observed. The effect of the enzyme supplement was in line with our findings, but the effects of SM on the production trais was a contrasting result compared to our findings. 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.

5.2.2. Nutrient digestibility, nitrogen retention, and metabolizable energy

Inclusion of SM in broiler diet and phytase supplementation enhanced faecal nutrient digestibility, metabolizable energy and N-retention of dietary treatments (Table 27). Sunflower meal significantly improved the absorption of crude fat, and increased AMEn, however, affected negatively starch digestibility. Using extra phytase supplementation improved all the measured parameters. Ravindran et al. (2001) 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. 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 27. Effects of dietary treatments on faecal nutrient digestibility, metabolizable energy, 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, − = without extra phytase supplementation; + = with extra phytase supplementation, AMEn—apparent metabolizable energy nitrogen corrected, ^{a,b} means with different superscripts in the same column are significantly different ($p<0.05$).

The effect of including SM at 15%, 20% and 25% with and without NSPase enzyme supplementation was evaluated on days 41 and 42. Sunflower meal and NSPase had no effect on the digestibility of DM, CP, and EE up to 20% level (Bilal et al., 2017). Three types of SM differing in the degree of dehulling were also used for broiler chickens between days 8 and 21. Dehulling resulted in CP levels of 37.4%, 40% and 42.5%, and CF levels of 21.7%, 20.2%, and 17.2%, respectively. Each meal was included in the diet at 20%. Birds fed SM with 42.5% CP, and 17.2% CF showed a significant increase in AME (Karkelanov et al., 2020).

In the current experiment birds fed SM (35.5% CP, 19% CF) showed an increase in AMEn, and fat digestibility even at higher level of CF content. The reason could be the age effect; older birds can tolerate and digest fibrous diet better than the younger birds. However, SM decreased starch digestibility may be due to the fibre diluting effect that hinder the efficiency of α-amylase (Tewelde et al., 2026a). Furthermore, SCFA are utilized by the intestinal enterocytes for growth and are transported to the liver to produce ATP. However, because of their low digestibility, NSP reduces the AME value of feed, and increase viscosity which adversely affects the digestibility of other nutrients (Aftab and Bedford, 2018).

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 (Hemati Matin et al., 2016). Importantly, enzyme supplement improved faecal nutrient digestibility of all nutrients including N-retention. Ravindran (2013) reported that benefits of enzymes generally diminish with age and responses are usually greater during the broiler starter phase compared with the finisher phase. This belief, however, is not always true; sometimes response may be greater during the finisher phase. The later agrees with the findings of the current study, that extra phytase improved nutrient digestibility and N-retention during the finisher phase. It was also 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).

Phytate has been reported in some studies to reduce energy utilization in poultry (Ravindran et al., 2001). In the study by (Fernandes et al., 2019) 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. As a reason for this improvement, the high doses of phytase may result in greater phosphorus release or restoration of the P/Ca balance, as well as lower residual phytate in the gut (Cowieson et al., 2011). In our research, although nutrient digestibility improved, this was not reflected in the production results. This is probably due to the already high efficiency of digestibility, as phytase enzyme supplementation was only able to slightly improve the excellent digestibility values.

5.2.3. Effects of dietary treatment on ileal amino acid digestibility

The ileal amino acid (AA) digestibility of the experimental diets is presented in Table 28. 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 arginine, lysine, methionine, threonine, valine, and tyrosine. Leucine was the only AA negatively affected by phytase supplementation. The reason for increase in digestibility could be that phytase increases apparent ileal amino acid digestibility in broilers chickens by removing the anti-nutritional effects of phytic acid.

Most published data on digestibility of AA in feed ingredients for poultry are based on excreta digestibility; however, ileal digestibility is the preferred alternative approach to estimate AA digestibility of feed ingredients (Lemme et al., 2004). The true digestibility coefficients of two high protein sunflower meals (40+® and 47®) have been established using intact and caecectomized birds. The mean digestibility for SM 40+® was higher compared with SM 47® in both intact birds (88.1% vs 85.7%) and in caecectomized birds (86.2% vs. 85.5%). The AA with the highest digestibility was arginine (96%) intact birds fed SM 40+® and the lowest was threonine (79%) in caecectomized birds fed SM 47® (Chobanova and Penkov, 2021). Other researchers who replaced the soybean-corn basal diet with 30% high protein SM also reported that, the highest apparent essential AA digestibility of high fibre SM fed to broilers was observed for arginine (89%) and the lowest for threonine (74.8%) (Waititu et al., 2018a). Similarly, in our experiment, the essential AAs with the highest digestibility were arginine and methionine (85%) and the lowest was threonine (58.9%) when SM was fed. Soybean replaced with SM at levels of 0%, 3%, 6%, and 9% with and without exogenous multienzymes at 100 mg/kg was fed during the grower (8–21 days) and finisher (22–35 days) phases. At the end of the trial the results of ileal digestibility of nutrients revealed that the birds fed 3% and 6% SM had a better CP digestibility compared with the C diet. At 9% SM level CP digestibility remain unaffected (Yaqoob et al., 2022). In the current study 30% SM had no effect in AA digestibility.

The standardized ileal AA digestibility value of feedstuffs for broilers was investigated, for SM, the AAs with the highest standardized ileal AA digestibility was Arginine (93%), and the lowest cystine (80%) followed by threonine (82%) (Lemme et al., 2004), but a bit higher than the SM contained diets of the current study. Their findings partially support our findings, and the higher percentage of digestibility could be the difference in apparent and standard ileal AA digestibility assays. Similarly, the standard ileal AA digestibility of SM for broiler chicks was found to be the highest in Methionine (91%) and arginine (91%) and the lowest was for threonine (73%) which fully agrees with our findings.

Table 28. 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.	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 ($p < 0.05$).

5.2.4. Length of the small intestinal segments as affected by the dietary treatments

The effect of phytase supplement and dietary treatments on intestinal segment length is shown in Table 30. Sunflower meal and the enzyme supplement failed to modify the length of the intestinal parts and the length of the whole intestine, except for ileum, which was significantly shorter when SM was fed.

Feeding SM at 15%, 20% and 25% with and without NSPase enzyme supplementation did not affect intestinal length (Bilal et al., 2017). Soybean replaced with SM at 3%, 6%, and 9%, with and without multienzymes (100 mg/kg) was fed during the grower (8–21 days) and finisher (22–35 days) phases. At 3% inclusion level the length of the duodenum and jejunum increased significantly, while other intestinal parts and ceca were unaffected (Yaqoob et al., 2022). These results broadly agree with the current findings. In contrast to our findings, soybean meal was replaced with dehulled SM at 33%, 66% and 100% levels with and without xylanase enzyme. SM increased intestinal length linearly, however, xylanase decreased the relative length of the small intestine (Al-molah et al., 2023).

Table 29. Effects of dietary treatments on the relative intestinal segment length (cm/kg BW)

Feed	**Phytase**	**Duodenum**	**Jejunum**	**Ileum**	**Whole small intestine**
C	-	12.89	31.21	32.84	76.94
	+	12.69	30.33	32.90	75.93
SM	-	11.75	30.41	29.50	71.67
	+	12.32	30.42	30.40	73.14
Feed	C	12.79	30.77	32.88^{a}	76.4
	SM	12.04	30.41	29.95^{b}	72.4
Phytase	-	12.32	30.81	31.17	74.3
	+	12.51	30.37	31.66	74.5
	SEM	0.24	0.48	0.61	1.03
P-value	Feed	0.119	0.722	0.018	0.060
	Phytase	0.696	0.663	0.683	0.913
	F x P	0.418	0.660	0.724	0.551

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 ($p<0.05$).

5.2.5. Dietary treatment effects on the relative organ weights

The effects of dietary treatments on the relative organ weights are shown in Table 31. 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 bursa of Fabricius. Replacing soybean meal with dehulled SM at 33%, 66% and 100% levels resulted a linear improvement in gizzard, proventricular and intestinal weight. Xylanase supplementation to these diets decreased the relative weights (Al-molah et al., 2023). Similarly, SM increased the relative gizzard weight in this experiment.

Table 30. Dietary treatment effects on the relative organ weight (%)

Feed	Phytase	Gizzard	Liver	Heart	Spleen	Bursa 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	
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, ^{a,b} means with different superscripts in the same column are significantly different ($p<0.05$).

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 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). Soybean replaced with SM at 3%, 6%, and 9%, with and without multienzymes (100 mg/kg) fed during the grower (8–21 days) and finisher (22–35 days) phases and had no effect on internal organ indexes of the heart, liver, gizzard, spleen, thymus but increased the weight of the bursa (Yaqoob et al., 2022). Their results agree with our findings, that enzyme increased the weight of the Bursa of Fabricus, and SM improved gizzard weight.

5.2.6. Dietary treatments effects on gut pH and jejunum histomorphology

The pH of the gut content results is presented in Table 32. Compared with the control, animals fed SM showed a significantly higher duodenal and jejunal pH, whereas caecal pH remained unaffected. Phytase supplementation failed to affect the gut pH.

Table 31. Dietary treatment effects on the pH of gut content (mean ± SEM)

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 ($p<0.05$).

In a soybean-wheat-maize based diet, soybean meal was replaced with SM to form a low fibre 30g/kg, medium fibre 40 or 50 g/kg during the grower (29 to 56 days), and high fibre 50 or 60 g/kg during the developer (57 to 112 days) was formulated and fed. On day 112, the influence of the crude fibre (CF) on relative weight of digestive organs was a linear improvement only for gizzard, no effect on relative length of duodenum, jejunum, ileum, the whole small intestine and caecum. However, the pH in the gastrointestinal segments such as gizzard, duodenum, jejunum, and ileum decreased linearly with increasing levels of CF from SM (Tüzün et al., 2021). Our results were found in line with their results in gizzard weight (Table 31) and intestinal segment length (Table 30) of our results. However, a contrasting result was recorded as SM increased duodenal pH ($p = 0.005$), and jejunal pH ($p < 0.001$) in the current study as shown in Table 32. The reason for the differences could be the age, type and amount of the CF content in the chicken's diet.

The histomorphology results are shown in Table 33. Feeding SM reduced VH and MLT but increased VW. Phytase supplementation reduced VH, VW, VH/CD, and VSA.

Table 32. Dietary treatments effect on the jejunal histomorphology

Feed	Phytase	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
	Phytase	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 ($p<0.05$).

In the study of Berwanger et al. (2017b) 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. Their findings for VH were consistent with the current study. Sunflower meal (0%, 4%, 8%, 12%, and 16% levels) with and without multienzyme supplement was fed to broilers from 1 to 21 days age. The contrasting result of this research was the multienzyme supplement enhanced intestinal morphology and led to better performance compared to diets without an enzyme, regardless of the level of SM included (Olivera et al., 2016). Longer villi and higher VH/CD ratio are generally correlated with better intestinal health and nutrient utilization (Tüzün et al., 2021). The reason for the contrasting could be the age of the birds, as the gut morphology development is faster in the younger age. In another study soybean was replaced with 0%, 10%, and 20% SM with and with NSPase, and fed to Ross-308 chicks from day 22 to 35. At the 20% inclusion level, SM reduced VH and VH/CD but had no effect on CD (Munawar et al., 2025).

Soybean meal was replaced with SM at 3%, 6%, and 9%, with and without 100 mg/kg multienzymes, during the grower (8-21 days) and finisher (22 to 35 days) phases. Results of jejunal morphology showed that 9% SM reduced VH and VH/CD ratio compared the non-supplemented C diet. Sunflower meal at 6 and 9% levels reduced CD (Yaqoob et al., 2022).The enzyme reduced VH, CD, and VH/CD, (Munawar et al., 2025) which fully agrees with our results.

5.2.7. Dietary treatments effects on the caecal SCFA contents

The effect of treatments on the short chain fatty acid (SCFA) content of caeca is shown in Table 29. The dominant SCFA was acetic acid, followed by butyric and propionic acid. Feeding SM did not affect SCFA contents, except for isovaleric acid. Phytase supplementation increased the amounts of acetic acid, propionic acid and butyric acid.

Table 33. Dietary treatment effects 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
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 ($p<0.05$).

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). Ross 708 male broilers were fed six diets, such as a corn-soybean meal-based diet as a positive control (PC), PC plus untreated SM (USM) and untreated wheat middling (UWM), and four additional diets in which USM and UWM were replaced with fibre degrading enzymes pre-treated SM and WM at a rate of 25%, 50%, 75% and 100%.

On day 42, the results showed that pre-treating SM and WM had not improved the SCFAs concentrations, except for lactic acid and Iso-butyric acid. The lactic acid concentration decreased linearly with increasing levels pre- treated SM and WM, and the opposite trend was observed for iso-butyric acid. However, no difference was observed in the concentrations of the total SCFAs among the treated groups, positive and negative controls (Njeri et al., 2023). In our experiment the addition of phytase increased the concentration of propionic acid, butyric acid and lactic acid. 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 (Tewelde et al., 2026a) which supports the current findings, except for isovaleric acid.

Short chain fatty acids 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 increased the concentrations for three of them. In a 42-day trial, 328 male Ross-508 broiler chicks were fed a wheat-based diets with and without xylanase. Broilers received a diet containing 16, 000 birchwood xylan units (BXU) per Kg of xylanase showed a notable change in cecal fermentation. The enzyme boosted acetate, propionate, and butyrate levels by over 20%, while slightly reduce the production of branched SCFAs Lee et al. (2017).

5.2.8. 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 caecal chyme (CC) (min: 1,148; max: 62,276). These sequences were assigned to 1072 OTUs at 97% similarity using the open approach.

5.2.8.1 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 3.). 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.

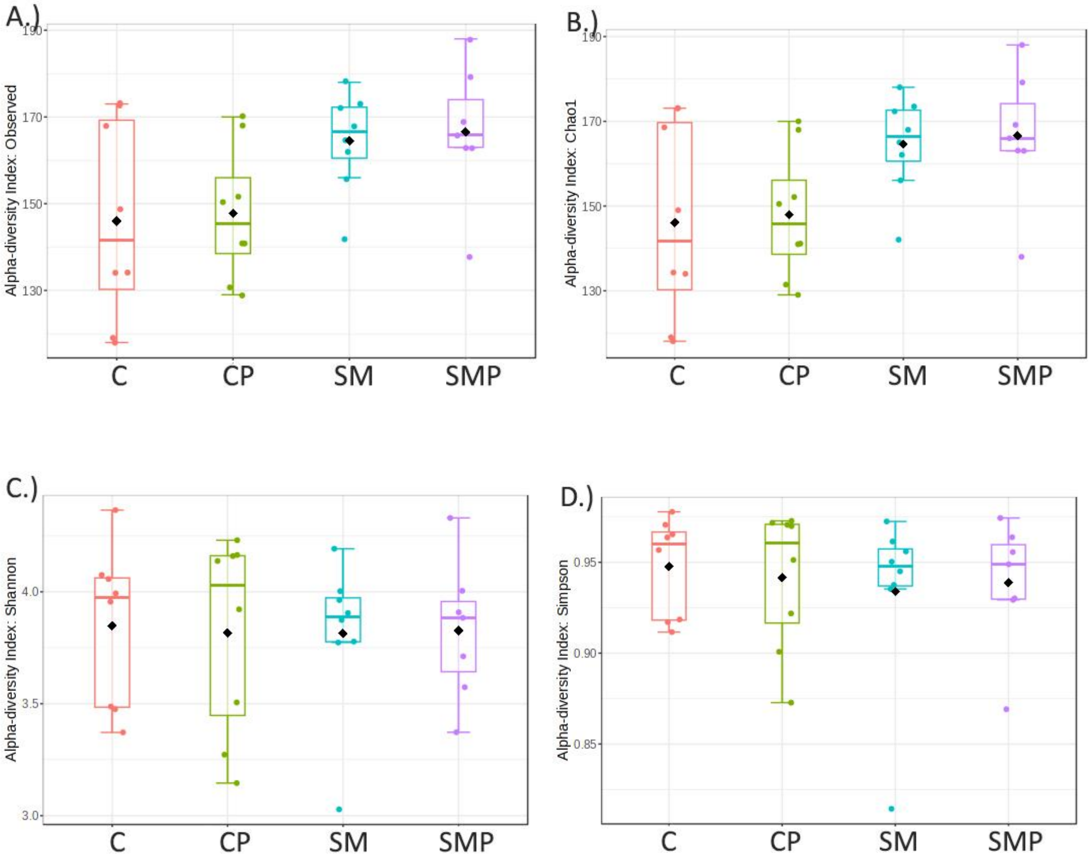

Figure 3.Alpha diversity indices of the caecal microbiota

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 4.). The highly significant difference in Unweighted UniFrac distance (p = 0.001, R^2 = 0.18) indicates that SM 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).

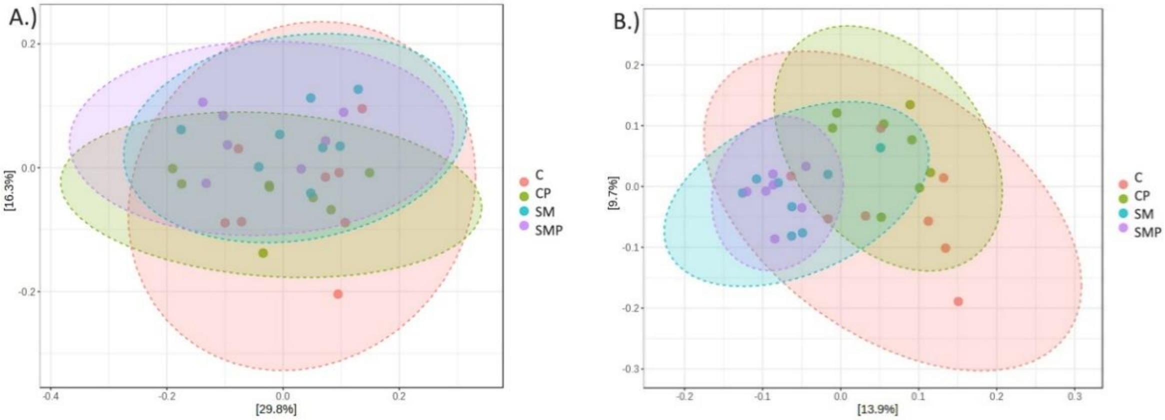

Figure 4. Beta diversity indices of the caecal microbiota

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)

5.2.8.2 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. (Table 34).

Table 34**.** Relative abundance of bacterial phyla in the caecal chyme of broiler chickens as affected by dietary treatments (%)

Phylum	**Diet**	**Phytase -**	**Phytase +**	**Mean**	**Feed**	**Phytase**	**Int.**
	C	94.86	95.72	95.29			
Firmicutes/Bacillota	SM	93.99	93.74	93.87	0.108	0.919	0.986
	mean	94.42	94.73				
	C	4.21	3.10	3.66			
Bacteroidetes	SM	3.44	3.05	3.25	0.541	0.624	0.684
	mean	3.83	3.07				
	C	0.61	0.72	0.67^{b}			
Tenericutes	SM	1.57	1.36	1.46^{a}	0.004	0.972	0.832
	mean	1.09	1.04				
	C	0.15	0.10	0.13^{b}			
Actinobacteria	SM	0.75	1.07	0.91^{a}	0.001	0.777	1.060
	mean	0.45	0.59				
	C	0.14	0.33	0.24			
Proteobacteria	SM	0.22	0.72	0.47	0.538	0.887	0.695
	mean	0.18	0.52				
	C	0.02	0.01	0.02			
Cyanobacteria	SM	0.03	0.05	0.04	0.528	0.843	0.651
	mean	0.03	0.03				
	C	0.00	0.01	0.00			
Not_Assigned	SM	0.01	0.01	0.01	0.432	0.652	1.818
	mean	0.00	0.01				

C= control, SM = sunflower meal, Phytase + = C or SM with extra phytase, Phytase – = C or SM without extra phytase

At the class level the relative abundance of the *Coriobacteriia* (C:0.12% SM:0.91%; p=0.001) and the *Mollicutes* (C:0.67% SM:1.47%; p=0.006) were significant higher in the treatment SM than in the C group. The dominant families were the two butyrate producing families, *Ruminococcaceae* and *Lachnospiraceae* (Table 35). Significant differences were found only in the case of some minor families (*Not assigned, Eggerthellaceae, Family_XIII*).

Table 35**.** Relative abundance of bacterial families in the caecal chyme of broiler chickens as affected by dietary treatments (%).

Family	Diet	Phytase -	Phytase +	Mean	Feed	Phytase	Int.
Ruminococcaceae	C	55.02	56.46	55.74	0.655	0.754	1.000
	SM	55.06	51.46	53.26			
	mean	55.04	53.96				
Lachnospiraceae	C	14.03	13.25	13.64	0.652	0.887	0.905
	SM	12.52	13.33	12.93			
	mean	13.27	13.29				
Clostridiales_vadinBB60_group	C	7.30	10.31	8.80	0.718	0.796	0.953
	SM	9.57	9.65	9.61			
	mean	8.43	9.98				
Lactobacillaceae	C	7.11	3.84	5.47	0.920	0.900	0.505
	SM	4.99	7.17	6.08			
	mean	6.05	5.50				
Christensenellaceae	C	5.90	6.16	6.03	0.906	0.903	0.970
	SM	5.86	5.94	5.90			
	mean	5.88	6.05				
Rikenellaceae	C	4.21	3.09	3.65	0.765	0.824	0.764
	SM	3.31	3.05	3.18			
	mean	3.76	3.07				
Erysipelotrichaceae	C	2.13	2.29	2.21	0.977	0.778	0.744
	SM	2.16	2.48	2.32			
	mean	2.15	2.38				
Veillonellaceae	C	1.03	0.53	0.78	1.005	0.903	0.775
	SM	0.80	0.77	0.78			
	mean	0.92	0.65				
Not_Assigned	C	0.86	0.96	0.91 ^{b}	0.030	0.923	0.761
	SM	1.62	1.59	1.61 ^{a}			
	mean	1.24	1.28				
Eggerthellaceae	C	0.15	0.10	0.12 ^{a}	0.002	0.835	0.791
	SM	1.02	1.07	1.05 ^{b}			
	mean	0.58	0.59				
Family_XIII	C	0.44	0.39	0.41 ^{b}	0.000	0.923	0.818
	SM	0.86	0.88	0.87 ^{a}			
	mean	0.65	0.63				

C= control, SM = sunflower meal, Phytase + = C or SM with extra phytase, Phytase – = C or SM without extra phytase

No significant treatment effects were found in the bacterial genera above 1% abundance (Table 36). However, the abundance of some minor genera was affected. Among them

Lachnospiraceae_UCG_010, Ruminococcaceae_*UCG_007,* Ruminococcaceae_*UCG_009, Ruminiclostridium_9* and *Ruminococcaceae_V9D2013_group* are well known butyrate producing families. Their abundance increased significantly in the SM groups.

Table 36. Relative abundance of bacterial genera in the caecal chyme of broiler chickens as affected by dietary treatments (%)

Genus	**Diet**	**Phytase -**	**Phytase +**	**Mean**	**Diet**	**Phytase**	**Int.**
Not Assigned	C	21.85	21.26	21.56	0.246	0.863	0.974
	SM	19.32	17.77	18.55			
	mean	20.59	19.52				
Ruminococcaceae_UCG_014	C	15.14	14.85	14.99	0.522	0.877	1.010
	SM	16.78	16.10	16.44			
	mean	15.96	15.47				
Ruminococcaceae_UCG_005	C	6.01	7.09	6.55	1.002	0.875	0.983
	SM	6.11	7.04	6.57			
	mean	6.06	7.06				
Christensenellaceae_R_7_group	C	5.80	6.07	5.94	0.919	0.901	0.988
	SM	5.38	5.81	5.60			
	mean	5.59	5.94				
Eubacterium_coprostanoligenes_group	C	4.23	4.72	4.47	0.327	0.943	0.824
	SM	3.51	2.75	3.13			
	mean	3.87	3.73				
uncultured_bacterium	C	4.19	4.53	4.36	0.956	0.869	0.923
	SM	5.02	3.86	4.44			
	mean	4.60	4.20				
Lactobacillus	C	7.11	3.84	5.47	0.925	0.916	1.575
	SM	4.42	7.17	5.79			
	mean	5.77	5.50				
Alistipes	C	4.21	3.09	3.65	0.753	0.912	0.872
	SM	3.43	3.05	3.24			
	mean	3.82	3.07				
Faecalibacterium	C	3.79	2.83	3.31	0.266	0.842	0.886
	SM	1.88	1.84	1.86			
	mean	2.84	2.34				
Ruminococcus_torques_group	C	3.10	2.78	2.94	0.432	0.858	0.890
	SM	3.13	4.86	4.00			
	mean	3.11	3.82				
uncultured_organism	C	0.87	2.44	1.66	0.786	0.940	0.970
	SM	1.79	2.61	2.20			
	mean	1.33	2.53				
Ruminococcaceae_NK4A214_group	C	1.66	2.33	1.99	0.761	0.827	0.954
	SM	1.53	1.78	1.65			
	mean	1.59	2.06				
Negativibacillus	C	2.18	2.25	2.21	0.325	0.834	0.830

	SM	4.11	2.84	3.47			
	mean	3.14	2.55				
	C	1.75	1.97	1.86			
Subdoligranulum	SM	2.20	1.31	1.76	0.935	0.837	0.897
	mean	1.98	1.64				
	C	1.03	1.76	1.39			
Ruminococcus_1	SM	0.92	0.89	0.90	0.310	0.925	0.916
	mean	0.97	1.32				
	C	1.13	1.75	1.44			
Ruminiclostridium_5	SM	1.48	1.40	1.44	0.995	1.087	0.897
	mean	1.31	1.57				
	C	2.40	1.73	2.06			
uncultured	SM	2.43	2.31	2.37	0.568	1.002	0.832
	mean	2.42	2.02				
	C	1.39	1.62	1.50			
Erysipelatoclostridium	SM	1.48	1.78	1.63	0.842	0.846	1.009
	mean	1.43	1.70				
	C	1.29	1.38	1.33			
Ruminococcaceae_UCG_010	SM	1.30	1.37	1.34	1.008	0.832	0.979
	mean	1.30	1.38				
	C	1.54	1.29	1.42			
Butyricicoccus	SM	1.10	0.96	1.03	0.422	0.850	0.990
	mean	1.32	1.13				
	C	0.75	0.95	0.85			
uncultured_Clostridia_bacterium	SM	2.04	1.57	1.80	0.268	0.907	0.845
	mean	1.39	1.26				
	C	0.12	0.12	0.12 ^{a}			
Eubacterium_hallii_group	SM	0.04	0.03	0.03 ^{b}	0.018	0.994	0.961
	mean	0.08	0.07				
	C	0.08	0.05	0.06 ^{a}			
Caproiciproducens	SM	0.03	0.02	0.03 ^{b}	0.028	1.377	0.858
	mean	0.05	0.04				
	C	0.12	0.13	0.12 ^{a}			
Lachnospiraceae_UCG_010	SM	0.06	0.08	0.07 ^{b}	0.041	0.840	0.974
	mean	0.09	0.10				
	C	0.29	0.47	0.38 ^{a}			
Blautia	SM	0.17	0.09	0.13 ^{b}	0.049	0.857	0.970
	mean	0.23	0.28				
	C	0.01	0.01	0.01 ^{b}			
Ruminococcaceae_UCG_007	SM	0.17	0.11	0.14 ^{a}	0.000	1.475	1.009
	mean	0.09	0.06				
	C	0.00	0.00	0.00 ^{b}			
Gordonibacter	SM	0.09	0.08	0.08 ^{a}	0.000	0.887	0.952
	mean	0.05	0.04				
	C	0.04	0.03	0.04 ^{b}			
Peptococcus	SM	0.16	0.12	0.14 ^{a}	0.005	0.881	0.839
	mean	0.10	0.07				

	C	0.03	0.02	0.02 ^{b}			
Ruminococcaceae_UCG_009	SM	0.08	0.08	0.08 ^{a}	0.045	0.867	0.983
	mean	0.05	0.05				
	C	0.10	0.12	0.11 ^{b}			
Family_XIII_UCG_001	SM	0.58	0.61	0.59 ^{a}	0.000	0.895	0.978
	mean	0.34	0.36				
	C	0.08	0.06	0.07 ^{b}			
Ruminiclostridium_9	SM	0.69	0.60	0.64 ^{a}	0.004	0.862	0.987
	mean	0.38	0.33				
	C	0.13	0.08	0.11 ^{b}			
CHKCI002	SM	0.65	0.99	0.82 ^{a}	0.006	0.806	0.851
	mean	0.39	0.54				
	C	0.02	0.02	0.02 ^{b}			
Ruminococcaceae_V9D2013_group	SM	0.07	0.04	0.05 ^{a}	0.005	1.690	1.128
	mean	0.04	0.03				

C= control, SM = sunflower meal, Phytase + = C or SM with extra phytase, Phytase – = C or SM without extra phytase

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. Feeding SM or using extra phytase did not result significant differences in the composition of the main bacterial phyla, but feeding SM increased the ratio of Tenericutes and Actinobacteria in the caeca. Similarly at class, order family and genus level only some minor bacterial groups changed significantly.

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 (Engels et al., 2016; Liu et al., 2021). The expansion of *Lachnospiraceae_UCG-001* likely also contributes to the cecal butyrate pool through the butyryl-CoA: acetate CoA-transferase (BCoAT) pathway. This enzyme allows the conversion of acetate into butyrate (Meehan and Beiko, 2014). 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 (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. The expansion of Actinobacteria is consistent with the higher concentration of sunflower-derived phenolic compounds, as this phylum encompasses specialized genera capable of polyphenol biotransformation (Alagawany et al., 2015b; Větrovský et al., 2014).

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 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 repertoire 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).

The significant increase in *Peptococcus* and *Family_XIII_UCG-001* suggests an adaptation to increased protein bypass. These taxa are known for their saccharolytic nature, primarily utilizing amino acids for fermentation (Whitman, 2015). This is likely linked to the high fiber content of sunflower meal, which is known to reduce proximal protein digestibility by shielding amino acids within the lignified plant cell wall matrix, it can shield proteins from proximal digestion, leading to a higher nitrogenous load in the cecum (Apajalahti and Vienola, 2016; Knudsen, 2014; Oliphant and Allen-Vercoe, 2019).

6. 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. Publications related to the PhD thesis

8.1.1. 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 (D1; 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 (Q4; IF: 0.7; CiteScore:1.1)

8.1.2. 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.

8.1.3. 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
2. **Kesete, G. T.,** Ferenc, H., Nikoletta, S., László, P., & Károly, D. (2024). Complex evaluation the dietary fiber effects in the broiler chicken nutrition. In *LXV. Georgikon Napok Tudományos Konferencia [65th Georgikon Days Scientific Conference] Keszthely, 2024. május 17–18.* (pp. 36–37).https://press.mater.uni-mate.hu/262/1/LXV-Georgikon-Napok-Tudomanyos-Konferenciakotet.pdf
3. Nikoletta Such , Ákos Mezőlaki , **Tewelde Kesete Goitom** , Brigitta Kiss , Laszlo Pal , and Karoly Dublecz. (2024): Effect of feeding extracted sunflower meal on intestinal content viscosity of laying hens. In: Pőr, Csilla; Szabó-Soós, Adrienn; Szabó, Péter (eds.) LXV. Georgikon Days Scientific Conference [65th Georgikon Days Scientific Conference] Keszthely, 17–18 May 2024. : Volume of abstracts, Keszthely, Hungary: Hungarian University of Agricultural and Life Sciences, Georgikon Campus (pp. 50-51).
4. Á. Mezőlaki , L. Pál , L. Wagner , MA Rawash , **KG Tewelde** , N. Such , K. Dublecz. (2023). Effects of feeding graded levels of sunflower meal on the apparent ileal amino acid digestion of pullets and laying hens. In: 23rd European Symposium on Poultry Nutrition - Book of Abstracts
   Rimini, Italy : World's Poultry Science Association (WPSA) (2023) 446 p. p. 364 Paper: PS5-006

8.2. Other publications in the field of animal nutrition

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

1. Such, N., Mezőlaki, Á., Rawash, M. A., **Tewelde, K. G**., Pál, L., Wágner, L., Schermann, K., Poór, J., Dublecz, K. (2023). Diet Composition and Using Probiotics or Symbiotics Can Modify the Urinary and Faecal Nitrogen Ratio of Broiler Chicken's Excreta and Also the Dynamics of In Vitro Ammonia Emission. ANIMALS, 13(3). http://doi.org/10.3390/ani13030332 **(Q1)**
2. Such, N., Schermann, K., Pál, L., Menyhárt, L., Farkas, V., Csitári, G., Kiss, B., **Tewelde, K. G.,** and Dublecz, K. (2023). The Hatching Time of Broiler Chickens Modifies Not Only the

Production Traits but Also the Early Bacteriota Development of the Ceca. ANIMALS, 13(17). http://doi.org/10.3390/ani13172712 **(Q1)**

3. Dublecz, K., Kiss, B., **Kesete, G. T**., Bartos, Á., Pál, L., Magyar, M., Benedek, Z., Such, N. (2023). Az ammónia emisszió csökkentésének takarmányozási lehetőségei a baromfi ágazatban. ÁLLATTENYÉSZTÉS ÉS TAKARMÁNYOZÁS, 72(3), 231–245.
4. Kiss, B., Erdélyi, M., Such, N., **Tewelde, K. G**., & Dublecz, K. (2024). A takarmánygyártás során használt expandálás és granulálás hatása a baromfi és sertés takarmánykeverékekre. GEORGIKON FOR AGRICULTURE: A MULTIDISCIPLINARY JOURNAL IN AGRICULTURAL SCIENCES, 28(Suppl. 2), 89–96.
5. Farkas, V., Mayer, A., Poór, J., Farkas, E. P., **Tewelde, K. G**., Kiss, B., Such, N., Pal, L., Csitari, G., Dublecz, K. (2025). Even Low Amounts of Amorphous Lignocellulose Affect Some Upper Gut Parameters, but They Do Not Modify Ileal Microbiota in Young Broiler Chickens. ANIMALS, 15(6). http://doi.org/10.3390/ani15060851(**Q1**)
6. Such, N., Rawash, M. A., Farkas, V., Poór, J., **Tewelde, K. G**., Pál, L., Csitari, G., Wagner, L., Farkas, E.P., Dublecz, K. (2025). Research note: Complex evaluation of whole oats and dehulled oats as feedstuffs for broiler chickens. POULTRY SCIENCE, 104(7). http://doi.org/10.1016/j.psj.2025.105207 **(D1)**
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9. SUMMARY

The demand for poultry products is increasing in line with the rapid increase in the world's population. It also means that more cereal grains and protein sources are needed for human nutrition, and more industrial by-products will be used in animal nutrition. Sunflower meal (SM), the by-product of the oil industry, is available in high amounts. However, its high fibre content is a limiting factor. In the frame of this work two feeding trials were carried out with broiler chickens and the responses of animals evaluated.

Two experiments were conducted. In the first experiment and two different SMs were used having a lower (CF: 10.35%; LFSM) and higher (CF: 16.68%; HFSM) crude fibre. Beside a corn, wheat and soybean meal-based control diet LFSM and HFSM were fed at 20 and 30% inclusion rates in the grower (day 11- day 24) and finisher (day 25- day 38) phases. After the end of the grower (days 24-27) and finisher periods (days 38-41) digestibility trials were also carried out in balance cage, using TiO_2 as an inert marker. The aim of the first experiment was to investigate the effects of feeding high fiber (HFSM) or low fiber (LFSM) containing SM compared with corn-wheat-soybean meal-based control diet (C) on the production traits, carcass composition, nutrient digestibility, gut content viscosity and caecal short chain fatty acid (SCFA) contents. The ileal digestibility of amino acids was determined also from different gut segments (distal jejunum, proximal ileum, distal ileum). The second experiment was aimed to examine whether extra phytase supplementation to SM-based diet could further improve the studied parameters. In this trial 4 diets were fed. A control diet without SM and diets containing SM at 20% in the grower and 30% in the finisher phases. Both, the control and the SM containing diets were fed with and without extra phytase (Quantum Blue, 1500FTU). After the finisher phase again a digestibility trial was carried out and the effects of SM and extra phytase evaluated. Faecal nutrient digestibility, ileal amino acid digestibility, the weight of gizzard, caeca, liver, heart, spleen, Bursa of Fabricius, the length of the different small intestinal parts, the pH of gut contents, the SCFA content of caeca, the histomorphometry of jejunum and the microbiota composition of the caecal content were determined.

In the first trial body weight gain (BWG) of chickens in the grower phase was not affected by SM, but in the finisher phase, the highest BWG was registered in the 30% high-fibre SM group, and the lowest gain belonged to the 30% low-fibre treatment. The carcass composition and the short-chain fatty acid composition of the caeca were not affected. Sunflower meal treatments did not affect the relative carcass, breast meat, and thigh weights. The abdominal fat percentage, however, increased when the LFSM-containing diets were fed. The low-fibre SM increased significantly the faecal digestibility of fats and AMEn at both age categories. However, decreased starch digestibility. The inclusion of HFSM and LFSM at 30%, reduced the total N excretion of chickens significantly at day 27. 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. 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.

Ileal AA digestibility on day 27 increased by feeding HFSM compared with the control group. The difference between the HFSM and control group was significantly higher for ARG, ILE, THR, TYR and CYS. Leucine was the only amino acid of which digestibility decreased if HFSM was fed. Feeding low fibre sunflower meals, 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 diet. On day 41, the inclusion of LFSM at 20% and 30% significantly enhanced the ileal digestibility of all amino acids ($p < 0.001$) except that of LEU. Both HFSM treatments increased the digestibility of LYS, THR, and TYR. Feeding HFSM30 diet further increased ARG and ILE digestibility. In the case of dietary treatment and age evaluation, apart from LEU, LFSM enhanced the ileal digestibility of all AAs in comparison with the control. However, HFSM decreased the absorption rate of LEU and resulted increase in the case of ARG, ILE, LYS, THR, VAL and TYR. The age of chickens resulted in a higher significant difference only in the case of MET and TYR digestibility on day 41. Distal jejunal AA digestibility on day 41 showed that feeding HFSM at both inclusion rates increased the digestibility of THR and TYR digestibility. High fibre SM at 20% level increased ILE while HFSM30 increased ARG and MET digestibility. The effect of LFSM in this gut section was low; the differences were significant only for MET (LFSM20, LFSM30), THR (LFSM20) and TYR (LFSM30).

Regarding the feed and intestinal segment interactions, 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, but except VAL and TYR no difference was observed between the proximal and distal ileal digestibility. The AA dynamics results showed 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 in the jejunum. The fastest absorbed AAs were ARG, LEU, MET and PHE. The disappearance rates of ILE, LYS, THR, VAL, TYR and CYS were slower. This suggests a difference in absorption kinetics prior to ileal completion. Feeding HFSM at 30% resulted in the lowest viscosity at both age groups. The age and feed interaction effect on digesta viscosity showed that growers had a significantly lower viscosity an indication that viscosity increases with age. The average viscosity at day 41 was significantly, 13.89% higher than that at day 27. The reason for this could be the higher feed intake of the older animals. The SCFA content of the caeca was not influenced by the HFSM and LFSM treatments

Dietary inclusion of SM at 20% and extra phytase supplementation did not modify production performance during the grower phase. However, during the finisher phase, (SM at 30%) and the entire experimental period (days 0 to 36), SM improved BW, BWG, and FCR. Using extra phytase did not modify the production parameters. Sunflower meal significantly improved the absorption of crude fat, and increased AMEn, however, affected negatively starch digestibility. Importantly, using extra phytase supplementation increased faecal nutrient digestibility of all nutrients including N-retention. Moreover, feeding the SM containing diets did not modify AA digestibility; however, extra-phytase supplementation increased the digestibility of arginine, lysine, methionine, threonine, valine, and tyrosine. Leucine was the only AA negatively affected by phytase supplementation. Feeding SM did not affect SCFA contents, except for isovaleric acid. Phytase supplementation increased the amounts of acetic acid, propionic acid and butyric acid in the caecal contents. Sunflower meal and the enzyme supplement failed to modify the length of the intestinal parts and the length of the whole intestine, except for ileum, which was significantly shorter when SM was fed. 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 bursa of Fabricius. Compared with the control, animals fed SM showed a significantly higher duodenal and jejunal pH, whereas caecal pH remained unaffected. Phytase supplementation failed to affect gut pH. Feeding SM reduced villus hight (VH) and muscle layer thickness (MLT) but increased villus width (VW). Phytase supplementation reduced VH, VW, villus hight to crypt depth ratio (VH:CD), and villus surface area (VSA). Feeding SM increased bacterial diversity in the caeca, but the evenness and structural complexity of the microbial community remained stable across treatments. Sunflower meal and extra-phytase had no effect on the relative abundance of the major phyla.

In conclusion, the results show that the fibre content of the extracted SM has a significant effect on the production traits, nutrient digestibility, the excretion of the different N compounds, and the gut viscosity of broiler chickens. The SM effects are also age dependent, which should be considered in the diet formulation. Extra-phytase supplementation increase nutrient digestibility, N-retention, digestibility of most of the AAs, the amounts of acetic acid, propionic acid and butyric acid but reduced VH, VW, VH/CD, and VSA. Therefore, HFSM and LFSM with and without extra phytase supplementation didn't cause adverse effect but increased most of the studied parameters. As a result, SM can be included up to 30% in iso-nitrogenous and iso-caloric broiler finisher diets without negative effects.

10. APPENDICES

A1: Bibliography

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